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REVIEW ARTICLE

Harnessing advanced extraction and profiling technologies for sustainable phytomolecule-based pest management

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Abstract

For decades, pest control has primarily relied on the use of synthetic chemicals. Although effective, this method has led to serious issues such as environmental pollution, prompting resistance in pests and posing health risks to beneficial organisms and even humans. Traditional methods used to extract plant-based compounds, such as maceration, Soxhlet extraction and hydrodistillation, are often outdated and inefficient, requiring large volumes of solvents and subjecting sensitive compounds to damaging heat. These ongoing challenges underscore the urgent need to seek safer, more sustainable solutions not only for managing pests but also for obtaining natural bioactive compounds. These methods are associated with high solvent consumption, poor selectivity, thermal degradation of heat-sensitive compounds and low recovery rates of active constituents, which limit the full potential of plant-derived bioactive compounds in pest management. Advanced extraction technologies are increasingly being adopted to overcome these challenges. Techniques such as ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction and pressurized liquid extraction employ innovative physical principles that enhance mass transfer, protect thermally sensitive bioactives and significantly improve extraction yields. These methods preserve the structural integrity and bioactivity of the compounds, making them highly suitable for further development. Additionally, modern analytical tools, such as high-performance liquid chromatography, mass spectrometry and metabolomic profiling, provide precise chemical characterization and quantification of the phytochemicals. The combination of advanced extraction techniques with chemical and metabolomic profiling ensures the high purity, efficacy and safety of phytomolecule-based insect-control agents. This review presents a novel extract-to-characterize framework integrating green extraction and metabolomic profiling to enhance phytochemical recovery and scalability of plant-based biopesticides.

Keywords: advanced extraction technologies; biopesticide formulations; eco-friendly pest control; metabolomic and chemical profiling; phytomolecule extraction

Introduction

Increasing concerns regarding the adverse effects of synthetic pesticides on human health and the environment have prompted a global shift toward sustainable pest management strategies. The global pesticide market, valued at $117.5 billion in 2024, is projected to reach $190 billion by 2029, with annual usage estimated to be between 2.0 and 3.5 million metric tons. Major consumers include China, the USA, Brazil and India. However, this rising use brings severe consequences, pesticides harm non-target species, disrupt ecosystems and pose serious health risks (Table 1). Chemicals are linked to cancer and neurological disorders, while persistent residues contaminate soil, water and the food chain. Alarmingly, pesticide exposure poisons 3 million people and causes 200,000 deaths each year, mostly in developing countries (1-4). These challenges have necessitated a reassessment of conventional pest control approaches and have fostered interest in eco-friendly alternatives, aligning with Integrated Pest Management (IPM) principles (5, 6). Among these alternatives, plant-based biopesticides have garnered significant attention. Derived from naturally occurring phytochemicals, they are recognized for their biodegradability, specificity to target pests and minimal ecological disruption (Table 2). Active compounds such as alkaloids, terpenoids, flavonoids, saponins and phenolics exert pesticidal effects through diverse mechanisms, including antifeedant, repellent, growth regulation and toxicity against insect pests, phytopathogens and nematodes (7). Importantly, the multifaceted composition of plant extracts reduces the likelihood of resistance development in pests, ensuring their prolonged efficacy. Globally, biopesticide production exceeds 3,000 tons annually, with market forecasts projecting a

Table 1. Common chemical pesticides usage and their side effect (3, 4)

Pesticides/ class	Common usage	Side effects
Organophosphates (e.g., Malathion, Parathion, Acephate, Chlorpyrifos)	Insecticides for crops, public health and livestock	Acute neurotoxicity (headaches, nausea, vomiting, muscle twitching, seizures); chronic neurodegeneration (Alzheimer's, Parkinson's); reproductive toxicity; cancer risk
Carbamates (e.g., Aldicarb, Carbaryl, Methomyl)	Insecticides, nematicides	Inhibit acetylcholinesterase; muscle weakness, dizziness, sweating, headache, nausea, nervous system depression, reproductive disorders, possible genotoxicity
Organochlorines (e.g., DDT, Endosulfan, Lindane)	Insecticides (some banned/ restricted)	Endocrine disruption, neurodevelopmental effects, cancer, reproductive toxicity, lipid metabolism disorders, persistent environmental contamination
Pyrethroid (e.g., Cypermethrin, Deltamethrin, Fenvalerate)	Household and agricultural insecticides	Neurotoxicity (tremors, headaches, fatigue), skin irritation, genetic damage, reproductive harm and possible cardiovascular effects
Glyphosate	Broad-spectrum herbicide (e.g., Roundup)	Skin, eye and respiratory irritation; suspected carcinogenicity; disrupts the shikimic acid pathway in plants and microbes
Paraquat	Non-selective herbicide	Severe oral and dermal toxicity, lung fibrosis, neurodegeneration (Parkinson's), multi-organ failure, skin ulceration
Soil Fumigants (e.g., 1,3-dichloropropene, Metam sodium, Chloropicrin)	Soil sterilization against nematodes, fungi, insects	Skin, eye and lung irritation; carcinogenicity; reproductive harm; increased premature birth rates in high-use areas
Fungicides (e.g., Azoxystrobin, Mancozeb, Captan)	Control of fungal diseases in crops	Skin, eye and respiratory irritation; some (e.g., Mancozeb) linked to thyroid and reproductive disorders
Rodenticides (e.g., Warfarin, Bromadiolone, Zinc phosphide)	Rodent control	Disrupt blood clotting (internal bleeding), nervous system effects, can be fatal to non-target species

Table 2. List of plant species exhibiting pesticidal properties along with their common name, family and utilized plant parts (14, 15)

Common name	Botanical name	Family	Utilized part(s)
Alexandrian Laurel	*Calophyllum inophyllum*	Clusiaceae	Seed oil
Apple of Sodom	*Calotropis procera*	Apocynaceae	Leaf paste
Bellyache Bush	*Jatropha gossypifolia*	Euphorbiaceae	Seed extract
Bergamot Mint	*Mentha citrate*	Lamiaceae	Essential oil
Bitter Lupin	*Lupinus termis*	Leguminosae	Seed extract
Black Pepper	*Piper nigrum*	Piperaceae	Oil, extract
Camphor Basil	*Ocimum kilimandscharicum*	Lamiaceae	Oil extract
Caraway	*Carum carvi*	Apiaceae	Fruit extract
Ceylon Oak	*Schleichera trijuga*	Sapindaceae	Seed oil
Chinaberry Tree	*Melia azedarach*	Meliaceae	Oil, extract
Cinnamon	*Cinnamomum aromaticum*	Lauraceae	Bark tissue
Clove	*Syzygium aromaticum*	Myrtaceae	Essential oil
Coconut	*Cocos nucifera*	Arecaceae	Coconut oil
Conyza	*Conyza dioscoridis*	Asteraceae	Flower extract
Coriander	*Coriandrum sativum*	Apiaceae	Seed oil, extract
Custard Apple	*Annona squamosa*	Annonaceae	Leaf tissue
Eastern Red Cedar	*Juniperus virginiana*	Cupressaceae	Essential oil
Eucalyptus	*Eucalyptus globulus*	Myrtaceae	Leaf paste, vapor
False Black Pepper	*Embelia ribes*	Myrsinaceae	Fruit extract, oil
Fennel	*Foeniculum vulgare*	Apiaceae	Fruit extract
Fenugreek	*Trigonella foenum-graecum*	Fabaceae	Seed extract
Field Bindweed	*Convolvulus arvensis*	Convolvulaceae	Leaf extract
Fish-Poison Tree	*Lonchocarpus spp.*	Leguminosae	Seed oil
Five-Leaved Chaste Tree	*Vitex negundo*	Lamiaceae	Leaf tissue
Garlic	*Allium sativum*	Alliaceae	Powdered clove
Guava	*Psidium guajava*	Myrtaceae	Leaf, leaf paste
Hiba Arborvitae	*Thujopsis dolabrata*	Cupressaceae	Extract
Hoary Basil	*Ocimum canum*	Lamiaceae	Leaf paste
Jimsonweed	*Datura alba*	Solanaceae	Leaf paste
Lemon/Orange	*Citrus spp.*	Rutaceae	Peel oil
Mahua	*Bassia longifolia*	Sapotaceae	Plant extract
Marigold	*Tagetes erecta*	Asteraceae	Root and stem
Mexican Tea	*Chenopodium ambrosioides*	Amaranthaceae	Fruit extract, oil
Mule Fat	*Baccharis salicifolia*	Asteraceae	Volatile oil
Mustard	*Brassica spp.*	Cruciferae	Leaf, flower extract
Neem	*Azadirachta indica*	Meliaceae	Oil, seed powder, leaf paste
Oil Palm	*Elaeis guineensis*	Arecaceae / Palmaceae	Oil
Pigeon Pea	*Cajanus cajan*	Fabaceae	Fixed oil
Pongam Tree	*Pongamia glabra*	Fabaceae	Oil, extract
Pyrethrum Daisy	*Tanacetum cinerariaefolium*	Asteraceae	Oil, powder
Rohitaka Tree	*Aphanamixis polystachya*	Meliaceae	Stem cortex, seed extract
Ryania	*Ryania speciosa*	Flacourtiaceae	Stem extract
Sesame	*Sesamum orientale*	Pedaliaceae	Oil
Sesame	*Sesamum indicum*	Pedaliaceae	Oil
Smartweed	*Polygonum hydropiper*	Polygonaceae	Leaf tissue
Soap Nut	*Sapindus trifoliatus*	Sapindaceae	Seed powder
Soybean	*Glycine max*	Fabaceae	Oil from seeds
Swallow Root	*Decalepis hamiltonii*	Asclepiadaceae	Root powder
Sweet Flag	*Acorus calamus*	Acoraceae	Oil, rhizome
Tobacco	*Nicotiana tabacum*	Solanaceae	Plant extract
Turmeric	*Curcuma longa*	Zingiberaceae	Powdered rhizome
Water Hyacinth	*Eichhornia crassipes*	Pontederiaceae	Leaf extract
White Lupin	*Lupinus albus*	Fabaceae	Seed extract
Wild Sage	*Lantana camara*	Verbenaceae	Whole plant extract

valuation of over $10 billion by 2027 (8, 9). Biopesticides account for only 4.2 % of pesticide use in India due to slow approvals, quality issues and limited farmer trust. Their frequent application and handling challenges make chemical pesticides a preferred choice under high pest pressure. Their adoption is increasing at an annual rate of approximately 10 %, driven by rising awareness and supportive regulatory frameworks despite ongoing regional limitations. However, the success of plant-based biopesticides largely depends on the efficiency of extraction, purification and formulation techniques. Traditional methods, such as maceration and Soxhlet extraction, are often hampered by low yields, long durations and degradation of heat-sensitive compounds. In contrast, modern extraction technologies provide substantial advantages, including improved yield, enhanced solvent efficiency and better preservation of bioactive integrity (10). Equally critical is the standardization of biopesticide formulations, as variations in plant metabolite profiles due to geographical, seasonal and methodological differences can compromise their field performance and reproducibility. Analytical techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS) and metabolomic profiling are instrumental in quantifying and characterizing active constituents, thereby ensuring the quality, stability and consistency of the final products (11, 12). Synthetic pesticides pose risks to humans, animals, beneficial insects and ecosystems. In contrast, botanical biopesticides offer eco-friendly manufacturing, are biodegradable, cost-effective and safe for humans and the planet. Easily washed-off produce leaves minimal residue. With the rising demand for sustainable farming, their role in pest control is more vital than ever (13). This review critically examines the increasing importance of plant-based biopesticides as environmentally sustainable alternatives to synthetic chemicals. This underscores the need for innovation in extraction technologies, analytical methods and standardization protocols to enhance their practical applicability and contribution to sustainable agriculture.

Botanical biopesticides: An overview

Botanical biopesticides are naturally derived plant compounds used to manage various agricultural pests, including insects, fungi and nematodes. Plant-based pesticides are classified into insecticides, fungicides, nematicides and herbicides based on their targets and modes of action. Insecticides are effective against pests, such as defoliators and sap-sucking insects, which threaten crop productivity (5, 7). The use of botanical biopesticides dates back to ancient agricultural systems, where traditional knowledge enabled the application of plant extracts for pest control in these systems. Plants such as neem, tobacco and pyrethrum are commonly employed for their natural pesticidal properties. These practices reflect early forms of sustainable and ecologically responsible pest management. The resurgence of interest in botanical pesticides stems from their favourable safety profiles for non-target organisms, including humans and their reduced environmental persistence compared to synthetic pesticides (14-17)). Several botanicals have gained commercial relevance owing to their efficacy and environmental compatibility. Neem (*Azadirachta indica*) is particularly notable for its broad-spectrum activity against numerous insect pests, primarily because of the presence of azadirachtin, which acts as a feeding deterrent, growth regulator and reproductive inhibitor (18). Botanical biopesticides like terpinen-4-ol, neem and rotenone offer targeted pest control by disrupting mitochondrial function or acting as nerve toxins, unlike broad-spectrum synthetics. They effectively manage pests such as *Lipaphis erysimi* while sparing beneficial insects like *Coccinellidae* and *Trichogramma*. Compounds like D-limonene and rotenone also show strong activity against insect larvae and nematodes, making them eco-friendly alternatives (14-16, 19, 20). When applied correctly, botanical biopesticides provide a safer, biodegradable and environmentally benign alternative to synthetic chemicals, aligning well with integrated and sustainable pest-management strategies.

Active phytochemicals and their mode of action

Phytochemicals derived from plants exhibit multifaceted effects on insect physiology and behaviour, positioning them as effective agents for eco-friendly pest management (15). These bioactive compounds are primarily classified into five major groups (alkaloids, terpenoids, flavonoids, phenolics and essential oils), each with distinct chemical structures, yet sharing the ability to disrupt critical insect and pathogen processes through insecticidal, antifungal, antifeedant and repellent activities (21). Alkaloids (e.g., nicotine and quinine) are nitrogen-containing bases that interfere with the insect nervous system by binding to acetylcholine receptors or inhibiting acetylcholinesterase. This leads to neuronal hyperexcitation, convulsions and ultimately paralysis, manifesting as strong insecticidal effects (22). Additionally, alkaloids exhibit antifungal properties by disrupting fungal membrane integrity and inhibiting ergosterol biosynthesis (23). Terpenoids, including monoterpenes (e.g., limonene and menthol) and sesquiterpenes (e.g., azadirachtin), act on insect membranes and ion channels, altering their fluidity and permeability (24). Azadirachtin interferes with ecdysteroid signaling, thereby impairing the molting and metamorphosis processes. Monoterpenes act on octopaminergic receptors, leading to repellent and antifeedant responses (25, 26). Moreover, terpenoids can exhibit synergistic antifungal activity by increasing membrane permeability, thereby enhancing the absorption of other bioactive agents (27). Flavonoids, a group of polyphenolic compounds, inhibit insect digestive enzymes, such as α-amylase and proteases, thereby reducing nutrient assimilation and stunting growth. Additionally, their UV-absorbing chromophores can generate reactive oxygen species upon light exposure, causing oxidative damage to both insect pests and fungal pathogens. As antifeedants, flavonoid glycosides impart bitterness, deterring herbivory even at sublethal concentrations (28, 29). Phenolics, including tannins and phenolic acids, form stable complexes with proteins in the insect gut, reducing enzymatic activity and nutrient bioavailability (30). Tannins also chelate metal ions, disrupting the microbial symbionts essential for digestion. Their antifungal activity stems from the inhibition of cell wall-degrading enzymes and suppression of spore germination (31). Essential oils, which are complex blends of volatile terpenoids and phenolics (e.g., thymol and carvacrol), function as fumigants and contact toxins. They penetrate the insect cuticle, disrupt membrane integrity and inhibit mitochondrial respiration in insects. Their volatility also confers strong repellent properties, effectively reducing host-seeking and oviposition behaviour (32, 33). Crucially, synergistic interactions among phytochemicals enhance their efficacy as pesticides. For instance, combinations of flavonoids and terpenoids can potentiate the inhibition of acetylcholinesterase, whereas phenolics may increase the uptake of alkaloids across insect membranes. Such synergism not only amplifies bioactivity but also mitigates the development of pest resistance by simultaneously targeting multiple physiological pathways.

Need for advanced extraction techniques

Conventional extraction methods, such as maceration, Soxhlet extraction and hydrodistillation, have several limitations in the efficient recovery of bioactive compounds from plant materials. These techniques often result in low yields, extended processing durations and simultaneous extraction of unwanted impurities. Moreover, many bioactive phytochemicals, including flavonoids, alkaloids and essential oils, are highly sensitive to high temperatures. Prolonged heat exposure during traditional methods can lead to thermal degradation, which compromises the chemical structure and biological activity of these molecules, ultimately reducing their effectiveness in practical applications. In addition to thermal sensitivity, conventional methods often lack precision and reproducibility. Variability in solvent polarity, plant matrix interactions and manual procedures contribute to inconsistent extraction outcomes. This inconsistency hinders the standardization of phytochemical compositions and poses a major obstacle to their commercial scalability for use in sustainable crop protection strategies (10-12, 34, 35) (Fig. 1). Therefore, the development and integration of advanced extraction technologies are essential for enhancing the recovery, stability and functional quality of plant-derived biopesticides.

Advanced extraction methods

Traditional extraction methods are time-consuming and costly, whereas advanced techniques like microwave-assisted and supercritical fluid extraction offer faster, higher-yield results with greater purity and less solvent use. Their scalability and eco-friendly nature make them more practical and cost-effective for commercial applications. Each method includes the principle, procedure, merits and applications.

Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction is an advanced technique that uses fluids above their critical temperature and pressure to selectively isolate bioactive compounds. Carbon dioxide (CO_2) is the most widely used supercritical fluid owing to its non-toxic nature, moderate critical parameters (31.1 °C and 73.8 bar) and environmental compatibility (36). In this process, CO_2 exhibits gas-like diffusivity combined with a liquid-like solvating capacity, allowing efficient penetration into plant matrices and enhanced recovery of target molecules without causing thermal degradation. The method begins with loading plant biomass into an extraction chamber, after which pressurized CO_2 is introduced. By precisely controlling the pressure and temperature, the solvating power of CO_2 can be tuned to optimize the extraction of specific compounds (Fig. 2). This is particularly beneficial for isolating non-polar bioactives, such as terpenoids, alkaloids and other lipophilic secondary metabolites (37, 38). Supercritical fluid extraction offers multiple advantages, including solvent-free extracts, minimal downstream processing, precise selectivity and effective preservation of heat-sensitive compounds. Furthermore, the recyclable nature of CO_2 contributes to the environmental sustainability of this technique (39). Importantly, the selective extraction of nonpolar compounds without residual solvent contamination represents a significant improvement over the conventional solvent-based method (40).

Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction is an advanced technique that employs microwave energy to heat solvents in direct contact with plant matrices, facilitating the efficient recovery of bioactive compounds. The underlying mechanism is based on dipole rotation and ionic conduction, in which microwave radiation induces rapid molecular movement. This results in effective cell wall disruption and accelerated mass transfer of the target molecules into the solvent phase (41, 42). In a typical MAE procedure, plant material is suspended in a suitable solvent and

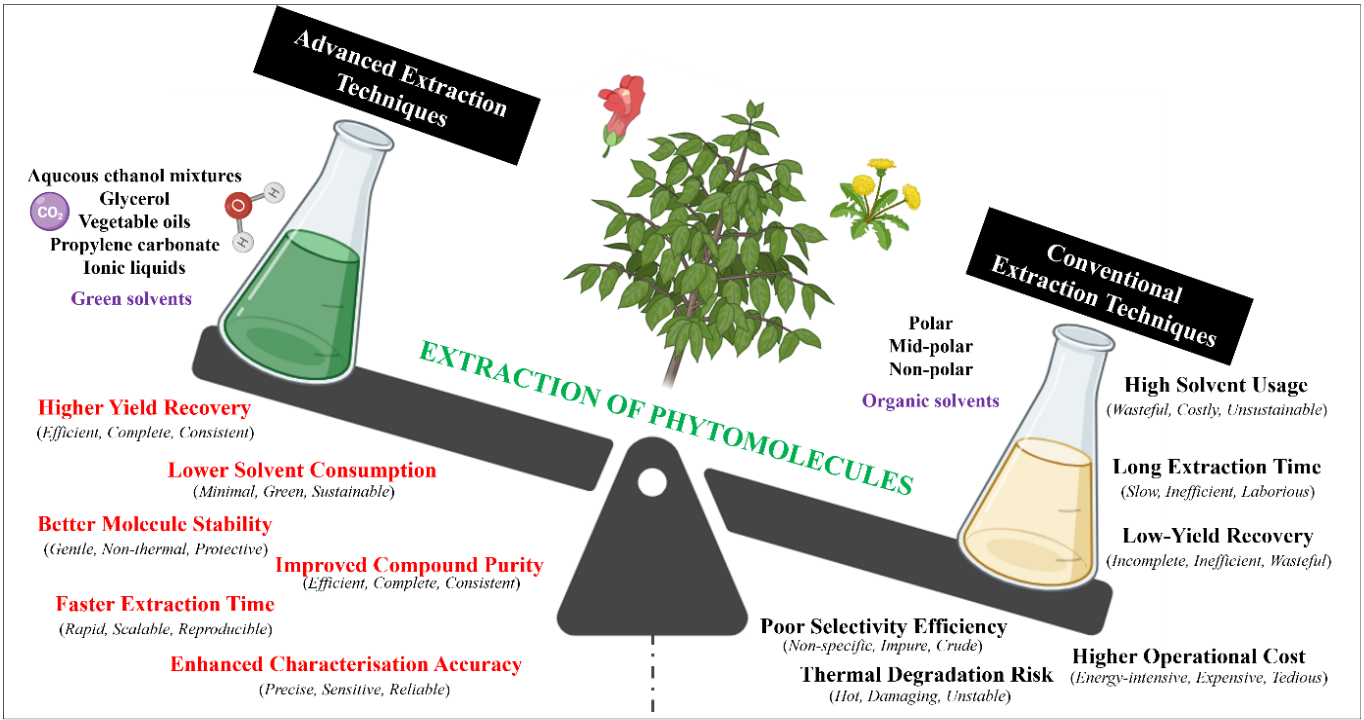

Fig. 1. Comparison of advanced and conventional techniques for phyto molecules extraction.

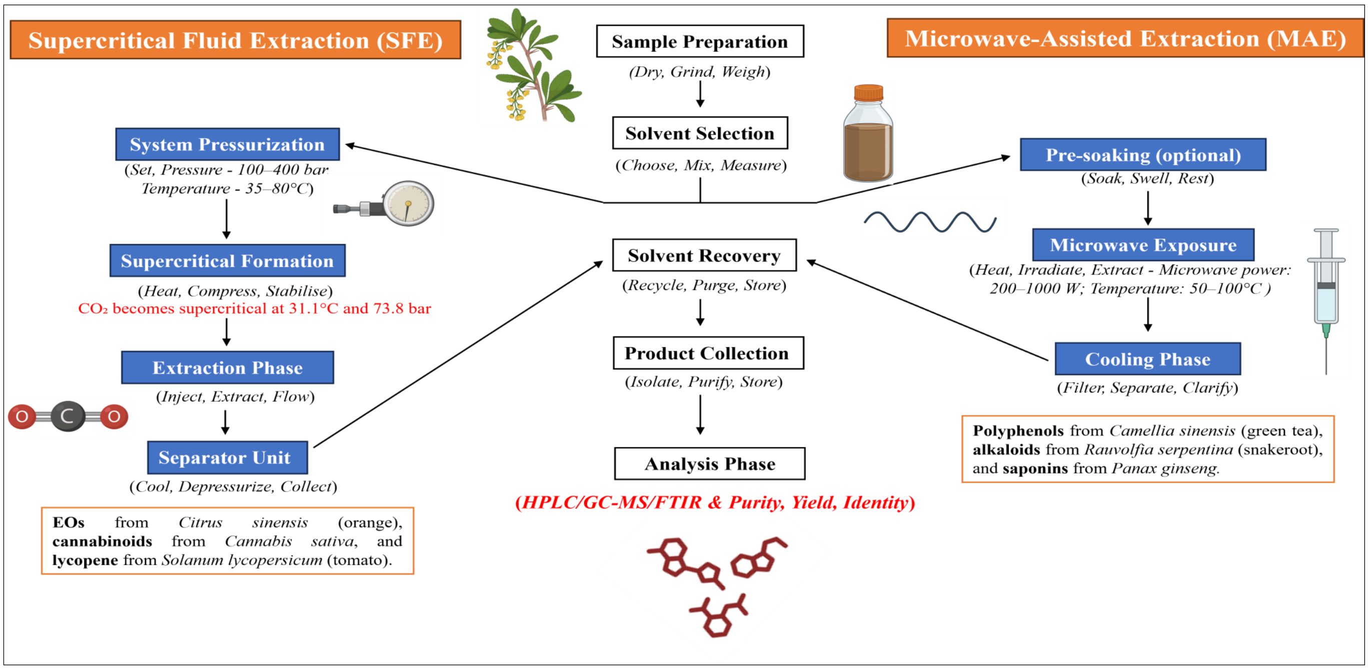

Fig. 2. Workflow of supercritical fluid and microwave-assisted extraction techniques.

subjected to controlled microwave irradiation (43). Key process variables, such as microwave power, irradiation time, extraction temperature and solvent polarity, are carefully optimized to maximize the yield while preserving the integrity of thermally sensitive compounds (37, 40, 44) (Fig. 2). Microwave-assisted extraction offers several advantages for biopesticide production. It significantly shortens the extraction time compared to traditional techniques, reduces the volume of solvent required and enhances the efficiency of extracting thermosensitive phytochemicals by enabling rapid heating under mild conditions (45). The selective heating effect also limits thermal degradation, thereby maintaining the biological activity of compounds that are crucial for pesticidal applications.

Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction is an advanced technique that employs high-frequency sound waves (20-100 kHz) to enhance the extraction of bioactive compounds from plant materials. The fundamental mechanism is based on acoustic cavitation, in which the rapid formation and collapse of microbubbles generate localized high temperatures and pressures. This mechanical effect disrupts plant cell walls, significantly improving solvent penetration and accelerating mass transfer (46, 47). The process involves immersing plant material in an appropriate solvent, followed by ultrasonic irradiation under controlled temperature and time conditions (Fig. 3). Critical parameters, such as ultrasound power, frequency, solvent type, solid-to-solvent ratio and extraction time, are optimized to maximize yield while ensuring the preservation of bioactivity (48, 49). Ultrasound-assisted extraction offers several advantages, including reduced extraction time, lower solvent consumption and enhanced recovery of thermolabile compounds owing to its ability to operate at lower temperatures. This technique is considered eco-friendly and economically viable for large-scale applications (47, 50). Most importantly, the UAE helps maintain the integrity of bioactive molecules, ensuring the continued efficacy of the extracted pesticides.

Pressurized Liquid Extraction (PLE)/Accelerated Solvent Extraction (ASE)

Pressurized Liquid Extraction (PLE), also known as Accelerated Solvent Extraction (ASE), is an advanced technique that utilizes elevated temperatures (typically 50-200 °C) and high pressure (10-15 MPa) to enhance the extraction efficiency of bioactive compounds from plant matrices (51). This technique offers significant advantages by disrupting cell walls and improving solvent penetration, thereby rapidly and effectively releasing intracellular secondary metabolites from the plant matrix. The process involves placing the sample into an extraction cell, filling it with an appropriate solvent, applying controlled pressure and temperature and collecting the extract. The choice of solvent and operational parameters is optimized based on the thermal stability and polarity of the target bioactive compounds (52) (Fig. 3). PLE/ASE offers several benefits, including reduced solvent consumption, shortened extraction times and compatibility with automation, making it ideal for the large-scale screening of plant-derived pesticides (53). The closed-system design minimizes solvent loss and environmental contamination, making this technique more consistent with green chemistry principles. The applications of PLE/ASE in biopesticide research are vast, especially in the isolation of thermally stable alkaloids, flavonoids, terpenoids and phenolic compounds with insecticidal or antifungal properties. However, optimization is essential to prevent the degradation of thermolabile compounds (7, 54). Critically, PLE provides a scalable and reproducible method with high extraction yields, which are crucial for the consistent formulation of biopesticide products.

Enzyme-Assisted Extraction (EAE)

Enzyme-Assisted Extraction (EAE) is a biotechnological method that utilizes specific cell wall-degrading enzymes, such as cellulases, hemicellulases and pectinases, to facilitate the release of intracellular bioactive compounds from plant matrices. The principle behind EAE lies in the targeted hydrolysis of complex polysaccharides in the cell wall, enhancing the bioavailability, extraction efficiency and specificity of the desired

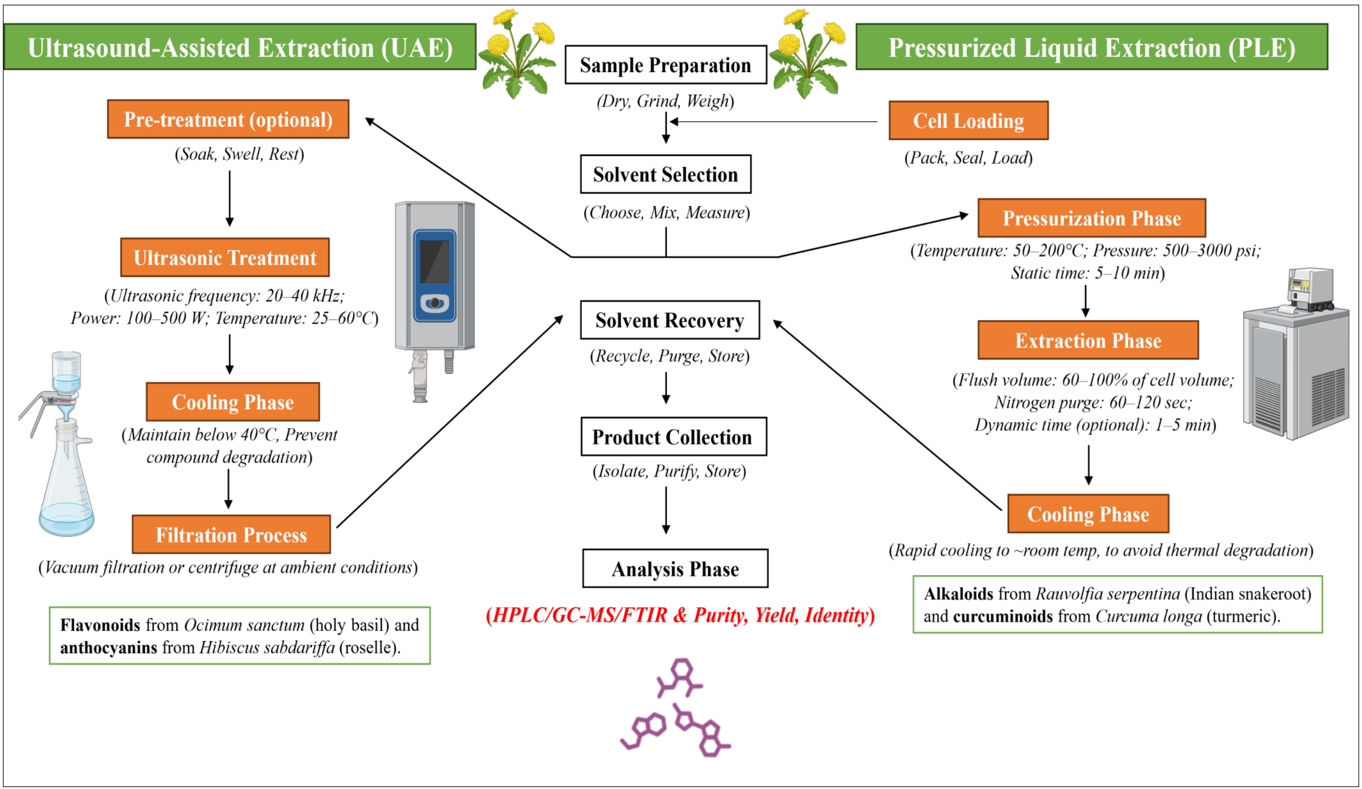

Fig. 3. Stepwise process of ultrasound-assisted and pressurized liquid extraction methods.

phytochemicals crucial for biopesticide formulations (55-57). The procedure involves pre-treating finely ground plant material with a defined concentration of enzymes under controlled pH, temperature and time conditions. Enzymatic action disrupts cell wall integrity, enabling the mild recovery of active secondary metabolites without causing chemical degradation (Fig. 4). The optimization of enzyme type, dose and reaction conditions is critical to maximize yield while maintaining the bioactivity of the extracted compounds (58, 59). EAE offers several benefits, including higher extraction efficiency, reduced solvent usage, preservation of thermolabile compounds and enhanced sustainability. Additionally, it allows for selective extraction, minimizing unwanted components that could interfere with biopesticidal activity (39, 40).

Ionic Liquid (ILs) and Deep Eutectic Solvent (DES)-based Extraction

Ionic liquids (ILs) and deep eutectic solvents (DESs) have emerged as green alternatives to conventional toxic organic solvents for extracting bio pesticidal compounds. These solvents are characterized by their tunable physicochemical properties, such as polarity, viscosity and hydrogen bond donation/acceptance, which allow for the selective dissolution and extraction of targeted bioactive molecules from plant matrices. ILs and DESs enhance the solubilization of structurally diverse secondary metabolites by disrupting plant cell walls via strong ionic interactions and hydrogen bonding. The general procedure involves preparing ILs or DESs from biocompatible components, followed by the direct extraction of plant materials under controlled temperature and agitation. Post extraction, simple techniques such as centrifugation and filtration are used to recover the active constituents (60-63) (Fig. 5). The key advantages of IL- and DES-based extraction include high extraction efficiency, thermal stability, non-volatility and reduced environmental impact compared to volatile organic solvents. Additionally, these solvent systems can be tailored by adjusting their constituents to enhance the selectivity for desired bio pesticidal compounds, thereby minimizing the co-extraction of unwanted impurities (63-65).

Subcritical Water Extraction (SWE)

Subcritical Water Extraction (SWE) is an eco-friendly extraction method that utilizes water at temperatures between 100 and 374° C under sufficient pressure to maintain its liquid state. Under these subcritical conditions, the dielectric constant of water decreases significantly, enhancing its solvating power for moderately polar and polar bioactive compounds. This alteration in the physicochemical properties of water allows for the efficient extraction of thermolabile and polar phytochemicals, which are essential for bio pesticidal formulations (66-67). The SWE procedure involves loading plant material into an extraction vessel, followed by the controlled introduction of water under elevated temperature and pressure. After the desired extraction period, the aqueous extract was collected, cooled and purified if necessary. Parameters such as temperature, pressure, flow rate and extraction time are optimized to maximize the yield and preserve the bioactivity of the target compounds (68-71) (Fig. 5). The key advantages of SWE include the elimination of toxic organic solvents, enhanced extraction efficiency, reduced processing time and minimal environmental impact (72, 73). Moreover, SWE preserves the structural integrity of sensitive bioactive molecules, which is critical for maintaining biopesticide efficacy.

Importance of stable pure bioactive components in biopesticide formulation

The stability of pure bioactive compounds, especially those isolated through advanced methods like supercritical fluid extraction, is crucial for formulating effective and consistent biopesticides. These techniques offer high selectivity and eco-friendly scalability, making them commercially viable despite

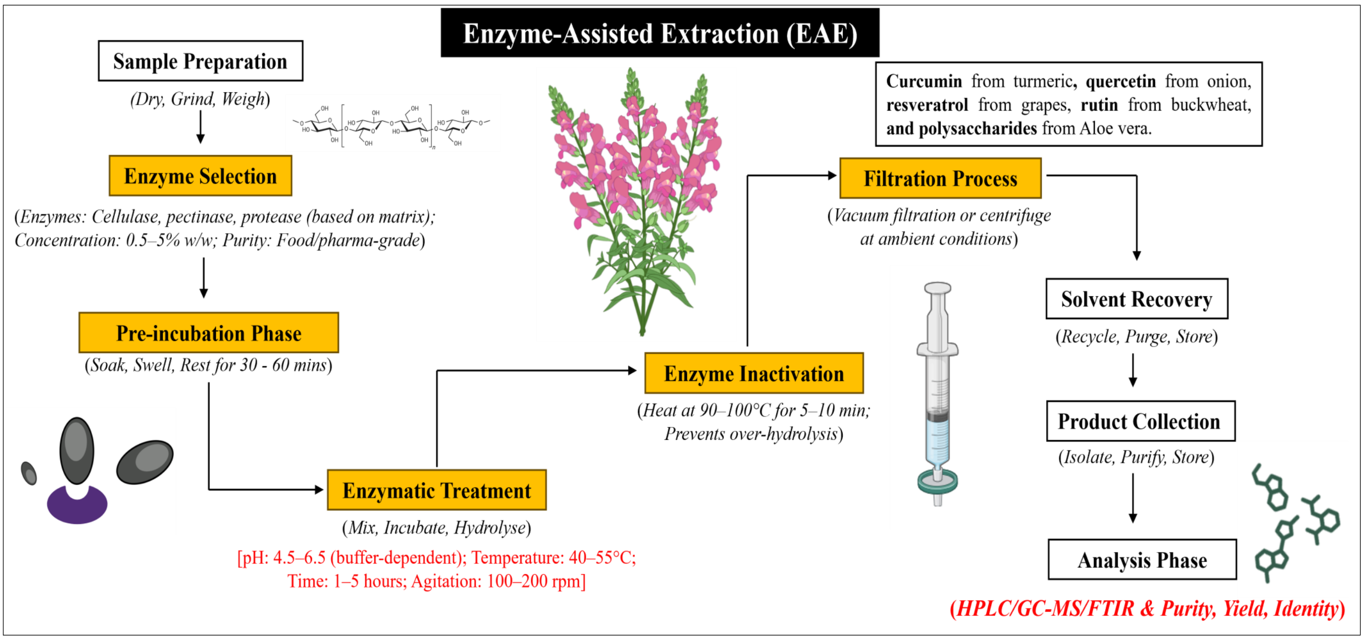

Fig. 4. Stepwise process of enzyme-assisted extraction method.

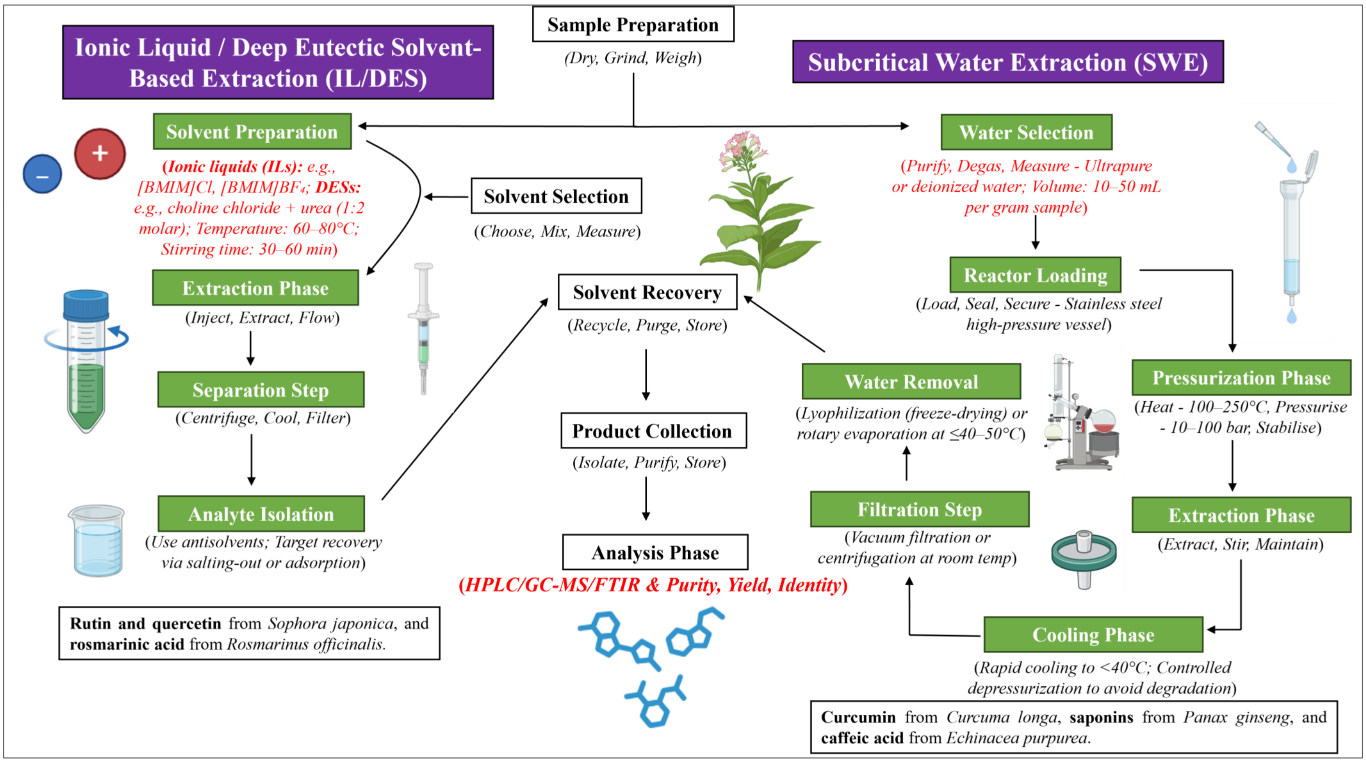

Fig. 5. Stepwise workflow of IL/DES-based and subcritical water extraction (SWE) techniques.

initial costs. These methods produce highly pure and stable compounds that are less prone to degradation during storage and handling, thereby significantly extending their shelf lives. Stable bioactive components preserve their biochemical integrity and biological activity, ensuring consistent and reliable pest-control performance. In addition, their stability enhances compatibility with carriers and adjuvants, facilitates controlled release, reduces variability, improves quality control and ensures regulatory compliance. Moreover, stable bioactive compounds degrade predictably, minimizing harmful by-products and reinforcing the eco-friendly nature of biopesticides (37, 74-76).

Modern analytical techniques

Modern analytical techniques, such as HPLC, MS and Metabolomic Profiling, have revolutionized biopesticide development by enabling the precise chemical characterization and quantification of phytochemicals. These methods are crucial for identifying and optimizing bioactive compounds in plant extracts that exhibit insecticidal properties (77-79). HPLC plays a key role in separating complex plant mixtures, isolating specific compounds of interest and quantifying them with high sensitivity and accuracy. This capability allows researchers to identify the exact bioactive molecules responsible for insecticidal activity, ensuring consistent formulation and potency of biopesticide products (80-82). MS complements HPLC by providing detailed molecular data, allowing researchers to confirm the identity of compounds based on their mass-to-charge ratio and fragmentation patterns. MS not only facilitates the identification of novel bioactive molecules but also provides insights into their chemical structures, helping elucidate their mechanisms of action against pests (80, 83, 84). Furthermore, MS enables the detection of trace compounds, ensuring that even low concentrations of insecticidal agents are monitored accurately. Metabolomic Profiling extends this analysis by examining the full spectrum of plant metabolites, including primary and secondary metabolites (85). Chemical fingerprinting of biopesticides is vital for isolating and quantifying the specific phytochemicals responsible for insecticidal activity. Identifying these compounds allows for the standardization and optimization of plant-based biopesticides, thereby enhancing their efficacy and safety. Moreover, fingerprinting ensures reproducibility, which is essential for regulatory approvals and quality control. This method also facilitates the assessment of variability in insecticidal compounds across different plant populations or environmental conditions, helping to identify the most potent sources for sustainable pest management strategies.

Market size and trend

The biopesticide market is experiencing rapid growth, driven by an increasing shift toward organic and eco-friendly farming practices (Fig. 6). Both farmers and consumers are becoming more aware of the detrimental effects associated with chemical pesticides, fostering a shift toward safer, natural alternatives. This increasing awareness has significantly contributed to market expansion. In 2023, the global biopesticides industry was valued at USD 7.3 billion and is projected to reach USD 23.74 billion by 2032, reflecting a robust compound annual growth rate (CAGR) of 14 % from 2024. A similar trend is emerging in India, where the demand for sustainable agricultural inputs is increasing. The Indian biopesticide market was estimated at USD 82.2 million in 2024 and is expected to grow to USD 204.1 million by 2033, with a CAGR of 9.23 %. This growth is fuelled by heightened public awareness of environmental safety and food quality, as well as government initiatives promoting biopesticides and restricting the use of synthetic chemicals in agriculture. Additionally, subsidies and incentives provided by the government are encouraging farmers to adopt these eco-friendly alternatives (11, 86-90).

Regulatory perspectives

The regulation of biopesticides varies globally, reflecting both their environmental benefits and the need for rigorous oversight to ensure their safety and efficacy. In the United States, biopesticides are regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). The Biopesticides and Pollution Prevention Division (BPPD) handles the registration process. Biopesticides are typically classified as reduced-risk pesticides, which require less data for registration than conventional pesticides, often resulting in approval within a year. However, genetically modified microbial pesticides are subject to additional regulatory scrutiny (91). In the European Union, biopesticides are governed by Regulation (EC) No. 1107/2009, which classifies them as plant protection products (PPPs). The approval process is a two-tier system, beginning with EU approval of the active substance, followed by individual member state approvals. Recent amendments have streamlined the approval process for microbial pesticides, reflecting an evolving regulatory approach (92). In India, the regulation of biopesticides is overseen by the Insecticides Act of 1968 and the Insecticides Rules of 1971, enforced by the Central Insecticides Board and the Registration Committee (CIBRC). Biopesticides receive provisional registration under Section 9(3B), with the option of extension for data generation before achieving permanent registration (11, 93). China regulates biopesticides under the "Regulations on the Administration of Pesticides," which ensures quality, efficacy and safety through specific registration procedures (94). In Japan, the Agricultural Chemical Regulation Law mandates a detailed registration process for biopesticides, including required studies overseen by the Ministry of Agriculture, Forestry and Fisheries (MAFF) (95). In Australia, the Australian Pesticides and Veterinary Medicines Authority (APVMA) ensures the safety and effectiveness of biopesticides through assessments based on the Agvet Code (96).

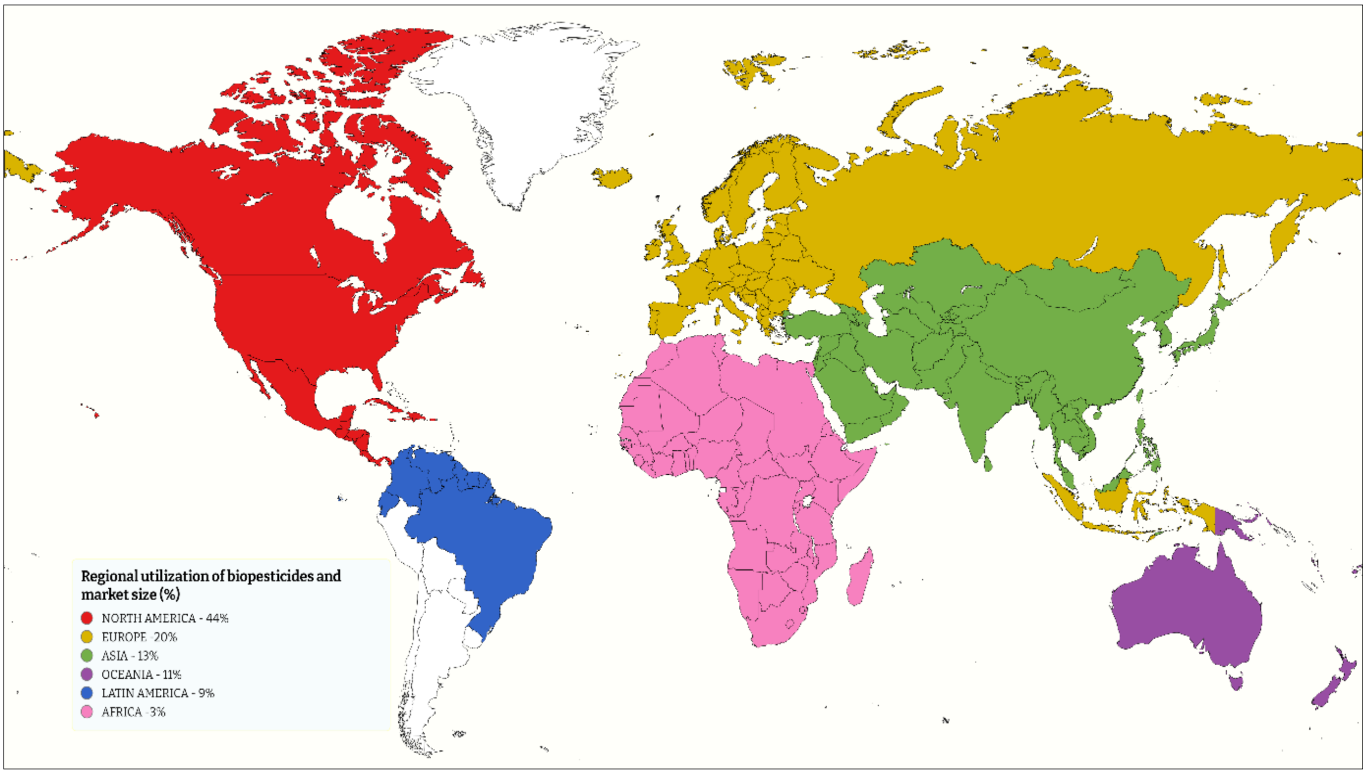

Fig. 6. Regional utilization of biopesticides and market size (%).

Barriers to commercialization and scalability

In India, commercialization of botanical extraction faces hurdles due to regulatory barriers, limited R&D and the high cost of advanced techniques like Supercritical Fluid Extraction, restricting small-scale producers (15). Addressing commercialization challenges in India's botanical extraction sector requires regulatory alignment, affordable technologies and farmer-focused innovations. Initiatives like NMITLI, blockchain traceability and climate-smart farming can enhance quality and global market access, transforming India's biodiversity into green export opportunities (11, 14). Labelling issues and regulatory ambiguity undermine user confidence and market perceptions of biopesticides, particularly in regions with limited knowledge of biological products. Startups are crucial for biopesticide innovation, focusing on new microbial strains and delivery systems; however, high research and regulatory costs limit scalability. Collaboration with larger companies and public bodies is essential to overcome these barriers. For biopesticides to effectively support sustainable agriculture and reduce reliance on conventional pesticides, harmonized regulations, standardized technical guidelines and innovation-friendly policies are necessary (11, 75, 97-100).

Challenges and future outlook

The advancement of green precision techniques for botanical extraction in sustainable biopesticide development holds substantial promise; however, several critical barriers hinder their widespread application in modern crop protection. One of the foremost challenges is scaling laboratory-optimized extraction methods to commercial production levels. The variability in raw plant materials, the complexity of multi-component phytochemical profiles and the sensitivity of active compounds to temperature, light and solvents result in inconsistencies in both yield and bioactivity. Techniques such as supercritical fluid extraction, microwave-assisted extraction and ultrasound-based methods, although efficient in laboratory settings, require high energy input, expensive instrumentation and finely tuned process conditions, making them less economically viable at scale. The lack of robust downstream purification and standardization protocols further compounds this issue, often resulting in formulations with reduced efficacy and shelf stability. Another pressing concern is the limited selectivity and environmental safety of several plant-derived biopesticides. Despite being perceived as eco-friendly, several botanical compounds exert non-specific toxicity, potentially harming beneficial arthropods, pollinators and soil microflora. The absence of a precise mechanism of action in many cases limits the ability to predict the ecological impact. Addressing this requires deeper molecular insights into plant-insect interactions, supported by omics-based tools such as metabolomics, transcriptomics and bioinformatics-guided structure–activity relationship studies. These approaches can aid in the identification of bio actives with high pest specificity and minimal off-target effects in the future. Further, microbial agents play a crucial role in insect control by targeting specific pests through pathogenic mechanisms. Combining microbial and botanical pesticides with nanoencapsulation enhances insecticidal synergy, stability and targeted delivery, offering a sustainable alternative to synthetic chemicals.

The incorporation of Artificial Intelligence (AI) and machine learning into biopesticide research has introduced a paradigm shift in process optimization and formulation design. Predictive modelling enables the rapid screening and optimization of extraction parameters, significantly reducing trial-and-error experimentation. When integrated with smart delivery systems, they respond to environmental triggers such as pH, enzymatic activity, or humidity. AI can facilitate real-time decision-making in the field, ensuring precise application and minimal waste of active ingredients. However, these technologies remain underutilized in agriculture because of their high developmental costs and the need for specialized technical expertise. Nanotechnology has emerged as a pivotal enabler in overcoming key formulation challenges. Encapsulating volatile and thermolabile phytochemicals within nanocarriers, such as chitosan nanoparticles, solid lipid nanoparticles, or mesoporous silica matrices, can significantly enhance their stability, bioavailability and controlled release. These nanostructures protect the active ingredients from degradation caused by UV radiation and microbial activity while enhancing foliar adhesion, systemic movement and bio efficacy under field conditions. Despite these technological strides, the full potential of biopesticides is curtailed by market, regulatory and socio-economic constraints. Higher production costs, limited availability and a lack of farmer awareness restrict their adoption, particularly among smallholder growers. Technical barriers related to storage; formulation uniformity and inconsistent field performance diminish farmers confidence. Additionally, the absence of standardized regulatory frameworks and international harmonization in biopesticide approval processes contributes to market fragmentation and delays in product commercialization in Brazil. Overcoming these systemic issues requires interdisciplinary collaboration, inclusive policy reform, public-private partnerships and capacity-building initiatives focused on farmer education and infrastructure development. Only through such integrated efforts can botanical biopesticides transition from niche innovations to mainstream tools in global crop protection.

Conclusion

The increasing demand for safer and more sustainable alternatives in agricultural insect control underscores the need for biopesticides. Phytomolecules derived from botanicals offer potent insecticidal properties and have minimal environmental impact. However, conventional extraction methods often fall short because of issues such as low efficiency, degradation of active compounds and lack of selectivity. In contrast, advanced extraction techniques provide significant improvements in the recovery, stability and preservation of bioactive compounds by utilizing controlled physical parameters. These innovations enable the development of precise, high-quality formulations that meet the practical demands of modern crop-protection systems.

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Authors' contributions

GK and KM were involved in literature collection, conceptualization, drafting and reviewing the manuscript. SM, MM and GKSA supervised the study and contributed to manuscript editing and finalization. VS, PG and KG assisted in manuscript drafting and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

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