Annals of Phytomedicine 15(1): 157-173, 2026

DOI: http://dx.doi.org/10.54085/ap.2026.15.1.14

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Review Article : Open Access

Phytotoxicology of botanical pesticides in medicinal crops: Mechanisms, multitrophic effects, and residue implications for high value phytomedicines

Chakati Santhosh*, Ettiappan Sumathi*◆, Sampathrajan Vellaikumar, Kannan Senguttuvan* and Alagarswamy Senthil*****

** Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India*
*** Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India*
**** Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India*

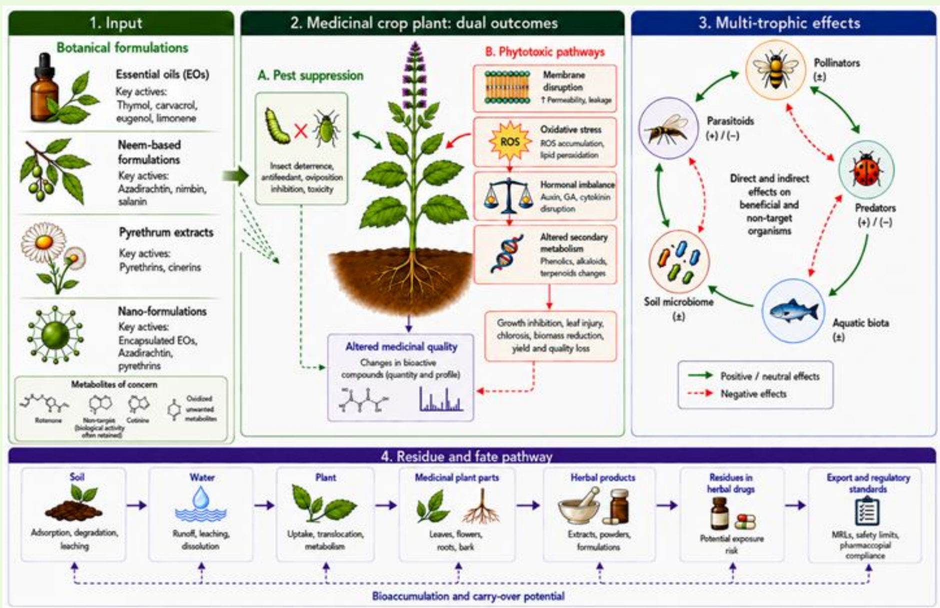
Article Info

Article history
Received 3 May 2026
Revised 7 June 2026
Accepted 8 June 2026
Published Online 30 June 2026

Keywords
Botanical pesticides
Phytotoxicity
Medicinal plants
Essential oils
Residue dynamics
Ecotoxicology

Abstract

Botanical pesticides are increasingly used in medicinal and aromatic plant (MAP) production as sustainable alternatives to synthetic pesticides due to their biodegradability, broad-spectrum activity and compatibility with organic and integrated pest management systems. However, growing evidence shows that many plant-derived compounds can cause significant phytotoxic, ecotoxicological and toxicological effects depending on formulation, concentration and exposure conditions. Major botanical groups, including limonoids, pyrethrins, rotenoids, alkaloids, and essential oil constituents, may disrupt membrane stability, photosynthesis, mitochondrial activity, redox balanceand hormonal signalling. These disruptions can alter the biosynthesis of alkaloids, terpenoids, phenolics and essential oils; thereby affecting medicinal quality, pharmacological efficacy and pharmacopeial compliance of MAPs. Low-dose exposure may stimulate defence-related secondary metabolism; whereas higher concentrations can induce oxidative and metabolic damage. In addition, the persistence of residues and degradation products, as well as their ecological impacts on beneficial arthropods and aquatic organisms, remains insufficiently understood. Advances in metabolomics, transcriptomics, nanoformulations, analytical chemistry and AI are improving understanding of phytotoxicity mechanisms and residue dynamics. Sustainable use of botanical pesticides; therefore, requires mechanistic phytotoxicological evaluation, standardised residue monitoring, ecological safety assessment and harmonised regulatory frameworks. This review provides a comprehensive framework for understanding the safe and sustainable use of botanical pesticides in medicinal crop production while maintaining medicinal quality and environmental safety.



Source: Made by us using Canva AI software

Corresponding author: Dr. Ettiappan Sumathi
Professor (Agricultural Entomology), Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India
E-mail: sumathi.e@tnau.ac.in
Tel.: +91-9865021683

1. Introduction

Medicinal and aromatic plants (MAPs) constitute a critical component of global healthcare systems and the phytopharmaceutical industry. Nearly 80% of the global population relies partly on herbal medicines for primary healthcare, while approximately 25% of modern pharmaceuticals are derived from or inspired by plant secondary metabolites (WHO, 2009; Newman and Cragg, 2020). Demand for herbal medicines, nutraceuticals and plant-based therapeutics has increased substantially due to growing consumer preference for natural healthcare products. High-value medicinal crops, including *Artemisia annua* L., *Catharanthus roseus* (L.) G. Don, *Mentha* spp., *Ocimum* spp., *Papaver somniferum* L., and *Withania somnifera* (L.) Dunal, are cultivated for pharmacologically important alkaloids, terpenoids, flavonoids, phenolics, and essential oils, whose composition and stability determine therapeutic efficacy and product quality (Bernhoft, 2010). However, production of medicinal crops is increasingly constrained by arthropod pests, phytophagous mites, fungal pathogens, nematodes, and weeds that reduce biomass and disrupt secondary metabolite biosynthesis (Shreedevasena *et al*., 2024).

Synthetic pesticides, including organophosphates, pyrethroids, neonicotinoids, carbamates, and triazole fungicides, have long been employed for pest management in MAP production systems. Nevertheless, growing concerns regarding pesticide residues, environmental contamination, ecological toxicity, and human health risks have prompted stricter regulatory standards for herbal medicinal products (HMPs) and phytopharmaceutical raw materials (WHO, 2007; European Medicines Agency, 2008). In medicinal crops, pesticide contamination is particularly critical because harvested plant materials are directly consumed or processed into therapeutic formulations. Consequently, even trace-level residues may compromise pharmacological safety, export compliance, and consumer confidence. These concerns have intensified interest in botanical pesticides as sustainable alternatives for medicinal crop protection. Botanical pesticides comprise insecticidal, acaricidal, fungicidal, nematicidal, and repellent compounds derived from plant secondary metabolites, including alkaloids, terpenoids, phenolics, pyrethrins, limonoids, and essential oils (Isman, 2006). Commercially important compounds include azadirachtin, pyrethrins, rotenone, nicotine, thymol, carvacrol, eugenol, limonene, and cinnamaldehyde (Pavela, 2016). Their adoption is further supported by perceived lower environmental persistence, reduced mammalian toxicity, and compatibility with organic and integrated pest management (IPM)-based production systems (Isman, 2006).

Despite these advantages, accumulating evidence indicates that botanical pesticides are not universally safe. Several plant-derived pesticidal compounds exhibit substantial phytotoxic, ecotoxicological, and toxicological effects depending on concentration, formulation, environmental conditions, and crop species (Dayan *et al*., 2009). Rotenone has been associated with mitochondrial dysfunction and neurodegenerative effects, whereas pyrethrins, nicotine, and concentrated essential oils may adversely affect pollinators, aquatic organisms, and beneficial arthropods (Bakkali *et al*., 2008). Botanical pesticides may induce diverse physiological and metabolic disturbances in medicinal plants, potentially affecting growth, stress responses, and phytochemical composition (Werrie *et al*., 2020). Unlike food crops, where yield reduction is often the primary concern, phytotoxicity in medicinal plants directly influences therapeutic quality because stress-induced metabolic alterations can modify alkaloid, terpenoid, phenolic, and essential oil composition. Dose-dependent hormetic responses are increasingly recognised in botanical pesticide exposure (Giunti *et al*., 2022).

Recent advances in analytical chemistry, omics technologies, environmental toxicology, and AI have substantially improved the understanding of botanical pesticide behaviour in medicinal agroecosystems. High-resolution chromatographic and spectrometric techniques now enable sensitive detection of botanical residues in complex herbal matrices, while metabolomics, transcriptomics, and proteomics provide systems-level insights into stress physiology and perturbations in secondary metabolism. Artificial intelligence (AI)- assisted modelling and computational toxicology are increasingly applied for phytotoxicity prediction, residue monitoring, and environmental fate assessment (Price and Watkins, 2003; Shaki *et al*., 2024). Nevertheless, knowledge regarding long-term ecological impacts, residue persistence, multi-trophic interactions, and regulatory harmonisation in medicinal crop systems remains fragmented. Therefore, this review critically evaluates the phytotoxicological, ecotoxicological, biochemical, environmental, analytical, and regulatory dimensions of botanical pesticide use in medicinal agroecosystems, with particular emphasis on phytotoxicity mechanisms, residue dynamics, ecological consequences, omics-based investigations, and emerging AI-driven approaches for toxicity and residue prediction. Given the increasing demand for residue-safe medicinal plant products, botanical pesticides are gaining importance as alternative crop protection tools. Unlike previous reviews that primarily focused on pesticidal efficacy, the present review specifically examines phytotoxicity mechanisms, medicinal quality implications, residue dynamics, ecological effects, and regulatory considerations in medicinal crop systems.

2. Major botanical classes used in medicinal crops and active phytochemicals

Botanical pesticides derive their activity from diverse plant secondary metabolites. Their chemical identity is central to mechanistic phytotoxicological assessment because pesticidal actions often parallel phytotoxic effects in non-target plants. Interest in plant-derived pesticides has increased due to their biodegradability, lower environmental persistence, and comparatively reduced toxicity to humans and beneficial organisms (Isman, 2006). These compounds include terpenes, alkaloids, flavonoids, polyphenols, quinones, amides, aldehydes, cyanogenic glycosides, thiophenes, saccharides, and polyketides. Many exhibit insecticidal, repellent, antifeedant, fungicidal, nematicidal, allelopathic, and insect growth regulatory activities (Isman, 2006; Pavela and Benelli, 2016). Their biochemical diversity enables multiple modes of action, reducing the risk of resistance development compared with synthetic pesticides.

2.1 Documented use in medicinal crops

Botanical pesticides have been applied in diverse MAP production systems, although comparative data across crops, pests, and formulations remain limited.Table 1shows the different botanical pesticides that are being used against different pests and diseases in medicinal cropping systems. Neem-based products are among the most extensively studied and have been evaluated in *Mentha* spp., *O. basilicum*, *W. somnifera*, *A. annua*, *P. somniferum*, and *Chamomilla recutita* L. (Isman, 2006; Pavela, 2016). Pyrethrin formulations have been investigated in lavender, valerian, and chamomile systems (Isman, 2006). Essential oil-based pesticides have also been studied in *Foeniculum vulgare* Mill., *Mentha piperita* L., *Coriandrum sativum* L., and related Apiaceae medicinal crops.

Table1: Botanicals used against pests and diseases of medicinal plants

Botanical source	Major active compound	Plant product used	Target pest/ disease	Medicinal crop	References
Azadirachta indica A. Juss.	Azadirachtin, nimbin, nimbecidine, salanine	Seed kernel extract	*Aphis gossypii*	Isabgol (*Plantago ovata*)	Patil *et al.*, 2011
Azadirachta indica A. Juss.	Azadirachtin, nimbin, nimbecidine, salanine	Seed kernel extract	*Schoutedenia emblica*	Indian gooseberry (*Emblica officinalis*)	Sridhar, 2011
Azadirachta indica A. Juss.	Azadirachtin, nimbin, nimbecidine, salanine	Neem oil	*Coccidohystrix insolitus*	Ashwagandha (*Withania somnifera*)	Ravikumar *et al.*, 2008b
Azadirachta indica A. Juss.	Azadirachtin, nimbin, nimbecidine, salanine	Proprietary neem products	*Leptocybeinvasa*	Eucalyptus nursery and field plantations	Kavitha Kumari *et al.*, 2009
Allium sativum L.	Allicin, diallyl sulfide, allyl cysteine sulfoxide	Essential oil	*Alternaria alternata*, *Fusarium* sp.	*Aloe vera* (L.) Burm.f.	Sharma *et al.*, 2010
Ocimum gratis-simum L.	Oxygenated hydrocarbon monoterpenes, sesquiterpenes	Leaf extract	*Pachliopta aristolochiae*	Shatawari (*Asparagus racemosus*)	Ramakrishna Mission, 2008
Tagetes erecta L.	Terpenone, lutein, thiophenes, limonene	Seed cake	*Meloidogyne incognita*	Coleus (*Coleus forskohlii*)	Seenivasan, 2011
Menthaarvensis L.	Cadiene, limonene, β-phellandrene	Leaf extract	*Petrobia harti*, *Tetranychus macfarlanei*, *Brevipalpus californicus*, *Bipolaris euphorbiae*	Oxalis, Ashwagandha, Mentha, Vasak	Ramakrishna Mission, 2008
Brassica juncea (L.) Czem.	Allyl isothiocyanate, benzyl isothiocyanate	Seed cake	*Meloidogyne incognita*	Coleus (*Coleusfors-kohlii*)	Seenivasan, 2011
Foeniculum vulgare L.	Terpenoids, oxygenated monoterpenes	Essential oil	*Phomopsis azadirachtae*	Neem	Fathima *et al.*, 2009
Madhuca indica J.F. Gmel.	Sapogenin, glucosidic saponin	Seed cake	*Cercosporar-auvolfiae*	Sarpagandha (*Rauvolfia serpentina*)	Arumugam *et al.*, 2010
Myristica fragrans Houtt.	Limonene, geraniol, myristicin	Essential oil	*Phomopsis azadirachtae*	Neem	Fathima *et al.*, 2009
Ocimum sanctum L.	Essential oil constituents	Essential oil	*Alternaria alternata*, *Fusarium* sp.	Aloe	Sharma *et al.*, 2010
Pongamia pinnata (L.) Pierre	Karanjin, pongapin, pongaglabol	Seed cake	*Cercosporarau volfiae*	Sarpagandha (*Rau-volfia serpentina*)	Arumugam *et al.*, 2010
Vigna unguiculata (L.)	Saponins, triterpenoids	Seed cake	*Meloidogyne incognita*	Coleus (*Coleus forskohlii*)	Seenivasan, 2011

Limonoids from *A. indica* (neem), particularly azadirachtin A and B, nimbin, nimbidin, and salannin, are among the most commercially used botanical insecticides globally (Isman, 2006). Azadirachtin is a highly oxygenated tetranortriterpenoid that acts as an insect growth regulator (IGR) by disrupting ecdysteroid and juvenile hormone titres, suppressing feeding, and inducing larval-pupal stasis (Mordue and Nisbet, 2000). Commercial neem products are generally standardised to azadirachtin content (0.03-3%), whereas crude neem seed kernel extracts (NSKE) contain the full limonoid complex and show broader biological activity. Their extensive use highlights limonoids as environmentally compatible alternatives to persistent synthetic insecticides (Lengai *et al.*, 2020).

Pyrethrins consist of six ester compounds extracted from dried flowers of *Tanacetum cinerariifolium*(Trevir.) Sch.Bip. (syn. *Chrysanthemum cinerariaefolium*). They act as modulators of axonic sodium channels, causing repetitive nerve firing and paralysis in insects (Casida, 1980). Although, rapidly photodegraded, pyrethrins remain highly toxic to arthropods, fish, and aquatic invertebrates. Synergisation with piperonyl butoxide (PBO) prolongs residual activity and toxicity (Weston *et al.*, 2005). Botanical synergists from plants such as *Anethum sowa* L. have also been explored to improve efficacy and reduce resistance (Handa and Dewan, 1974).

Rotenone, a rotenoid isoflavonoid from *Derris elliptica* (Wall.) Benth, *Lonchocarpus utilis* (A.C.Sm) A.M.G. Azevedo, and related legumes, inhibit mitochondrial Complex I (NADH:ubiquinone oxidoreductase), disrupting electron transport and inducing reactive oxygen species (ROS) generation (Caboni *et al.*, 2004). Its strong ichthyotoxicity and association with Parkinson's-like neurodegeneration have led to regulatory restrictions in several countries (Tanner *et al*., 2011). Similar inhibitory effects on plant chloroplasts and mitochondria contribute to its phytotoxicity (Møller, 2001). Concerns regarding mammalian toxicity and environmental persistence have greatly reduced its agricultural use (Isman, 2006).

Nicotine and related pyridine alkaloids, including nornicotine and anabasine from *Nicotiana tabacum* L., act as nicotinic acetylcholine receptor (nAChR) agonists, similar to synthetic neonicotinoids (Tomizawa and Casida, 2005). Although, largely replaced in commercial agriculture, nicotine-based preparations remain used in artisanal and organic farming systems, particularly in developing countries, where they pose occupational and ecological toxicity risks (Casida and Durkin, 2013). Nicotine is among several historically important phytochemical pesticides, alongside veratrum alkaloids, annonins, rocaglamides, and isobutylamides (Isman, 2006).

Essential oils are the most diverse and rapidly expanding class of botanical pesticides. These mixtures of monoterpenes, sesquiterpenes, and oxygenated derivatives are distilled from aromatic plants. Major constituents include thymol and carvacrol (*Thymus*, *Origanum*), eugenol and cinnamaldehyde (*Cinnamomum*, *Syzygium*), linalool and linalyl acetate (*Lavandula*, *Coriandrum*), menthol and menthone (*Mentha*), and terpinen-4-ol (*Melaleuca*) (Pavela and Benelli, 2016). Their mechanisms include membrane disruption, inhibition of acetylcholinesterase (AChE) and γ-aminobutyric acid (GABA)-gated neurotransmission, interference with octopaminergic signalling, and protein denaturation (Bakkali *et al*., 2008). These mechanisms may also induce phytotoxicity at high concentrations. Due to their rapid biodegradability and broad-spectrum activity, essential oils are increasingly explored as bioinsecticides, biofungicides, and bioherbicides (Pavela and Benelli, 2016). Advances such as nanoencapsulation, transcriptomics-guided screening, and bioinformatics-assisted formulation design are being developed to overcome volatility, hydrophobicity, and low residual persistence

Other important botanical actives include quassinoids such as quassin and neoquassin from *Quassia amara* L., alkamides including piperine and pellitorine from *Piper* spp., limonene and related citrus terpenes, and saponin-based products from *Quillaja, Saponaria,* Molina and *Yucca schidigera* Roezl ex Ortigies (Isman, 2006). Additional pesticidal plants, including *M. indica*, *Sapindus mukorossi* Gaertn., *Curcuma longa* L., *P. glabra*, *T. erecta*, and *Rheum emodi* D. Don, also exhibit promising pesticidal activity due to their diverse phytochemicals (Pavela, 2016; Lengai *et al*., 2020). Each botanical class possesses distinct phytotoxicological and ecotoxicological profiles requiring independent evaluation inMAP systems.

2.2 Formulation types and delivery systems

Formulation strongly influences the stability, penetration efficiency, bioavailability, and phytotoxicity of botanical pesticides (Regnault-Roger, 2012). Conventional formulations, including emulsifiable concentrates (EC), wettable powders (WP), soluble concentrates (SC), and dusts, are increasingly supplemented by advanced delivery systems. Despite their biodegradability and lower persistence, large-scale adoption remains constrained by instability, volatility, and inconsistent field efficacy (Kumar *et al*., 2025).

Nanoemulsions and nanocapsules represent major advances in botanical pesticide delivery. Droplet sizes below 200 nm enhance penetration of hydrophobic actives through plant cuticles and epidermal tissues, increasing both pesticidal efficacy and phytotoxic risk (Kah *et al*., 2018). Nanoformulations of thymol, carvacrol, and azadirachtin have shown improved efficacy in controlled studies (Natal *et al*., 2021; Chaudhary *et al*., 2017). However, phytotoxicological data for nano-botanical pesticides inMAPs remain limited. Enhanced cellular penetration may improve pest control while simultaneously disrupting intracellular metabolism in crop tissues at lower concentrations. Nanoencapsulation is also being explored to improve the stability and persistence of volatile essential oils under field conditions.

Microencapsulation using biodegradable polymers such as poly-lactic acid, chitosan, and zein enables controlled release of volatile essential oils. This approach reduces volatilisation and moderates plant exposure to concentrated actives. Chitosan-essential oil matrices are particularly relevant in MAP systems because chitosan itself acts as a plant defence elicitor, complicating phytotoxicological interpretation (Li *et al*., 2020). Slow-release wax-based formulations and emulsion-in-water systems developed for azadirachtin prolong residual efficacy (Johnson and Dureja, 2002), but may also increase residue persistence and plant surface exposure in harvested HMPs.

A major limitation in the literature is the lack of standardised application protocols accounting for crop stage, environmental conditions, and formulation-specific variables. Applications under unsuitable growth stages or atypical environmental conditions may overestimate or underestimate phytotoxicity, limiting cross-study comparability. Moreover, most evidence is derived from greenhouse studies, while field-based phytotoxicolo-gical data for MAP systems remain scarce.

3. Phytotoxicity mechanisms and crop physiology impacts

3.1 Types of phytotoxic effects documented for botanicals

Botanical pesticides induce both visible and sub-visible phytotoxic effects. Acute symptoms include chlorosis, necrosis, epinasty, leaf curling, tissue desiccation, growth inhibition, and reduced seed germination. Chronic effects involve disruption of photosynthesis, oxidative stress, hormonal imbalance, and altered secondary metabolism (Bakkali *et al*., 2008; Werrie*et al*., 2020). These effects are especially critical inMAPs, where therapeutic quality depends on specialised metabolites.

Essential oil-based botanicals are among the most common causes of phytotoxicity because volatile monoterpenes and phenylpropanoids readily penetrate plant tissues and disrupt cellular metabolism. Clove oil and eugenol caused severe membrane injury in broccoli and several weed species through electrolyte leakage and cuticular wax disruption (Bainard *et al*., 2006). Exposure of rice seedlings to β-pinene reduced chlorophyll content, protein accumulation, and carbohydrate metabolism, resulting in growth inhibition and poor seedling vigour (Singh *et al*., 2018). Foliar application of *Cymbopogon citrates* (DC.) Stap f and *Hyptis suaveolens* (L.) Kuntze essential oils caused dose-dependent reductions in chlorophyll pigments and photosynthetic activity in barnyard grass and rice, accompanied by membrane damage and cytogenetic abnormalities (Werrie *et al*., 2020).

In MAP systems, phytotoxicity commonly appears as interveinal chlorosis, leaf scorch, premature senescence, and wilting after repeated or high-dose applications. Monoterpenes such as thymol, carvacrol, menthol, and camphor destabilise membranes, causing electrolyte leakage and tissue collapse (Bakkali *et al*., 2008; Werrie*et al*., 2020). Citral, limonene, and farnesene also inhibit root growth, suppress lateral root formation, and reduce seed germination by interfering with hormonal regulation and microtubule organisation (Graña *et al*., 2016; Araniti *et al*., 2016).

Beyond visible symptoms, botanical pesticides induce oxidative burst, photosystem II (PSII) disruption, mitochondrial dysfunction, and altered phytohormonal signalling. These hidden responses modify alkaloid, phenylpropanoid, and terpenoid biosynthesis, thereby affecting medicinal quality even without external injury. Transcriptomic and metabolomic studies further show large-scale metabolic reprogramming under botanical stress, indicating that visual assessment alone cannot accurately determine crop safety (Werrie*et al*., 2020).

3.2 Cellular and molecular mechanisms

3.2.1 Membrane and organelle disruption

Disruption of membrane integrity is a primary phytotoxic mechanism of many botanical pesticides. Several monoterpenes also produce concentration-dependent cellular injury and tissue collapse in sensitive MAP species. Increased cytosolic Ca^{2+} influx further amplifies stress signalling and oxidative burst responses (Marcec*et al*., 2019). Biomimetic membrane studies showed that citronellal and citronellol modify membrane organisation without complete permeabilisation, whereas cinnamaldehyde interacts more strongly with membrane-associated receptors, indicating compound-specific phytotoxic mechanisms (Werrie *et al*., 2020).

Membrane destabilisation is closely linked with organelle dysfunction. Chloroplasts are highly susceptible because essential oils disrupt thylakoid membranes and photosynthetic electron transport. â-pinene inhibited PSII electron transport and damaged chloroplast membranes in spinach and *Cucurbita* systems (Kordali*et al*., 2008). Monoterpenes also uncouple mitochondrial respiration and oxidative phosphorylation, reducing adenosine triphosphate (ATP) synthesis and cellular energy availability (Abrahim *et al*., 2000). In tobacco BY-2 cells, 1,8-cineole caused severe disruption of mitochondria, nuclei, dictyosomes, and plasma membranes, highlighting organelle deterioration as a major component of botanical phytotoxicity (Werrie*et al*., 2020).

Monoterpene exposure additionally alters membrane composition by increasing unsaturated phospholipid fatty acids and modifying sterol profiles, particularly stigmasterol accumulation (Werrie *et al*., 2020). These changes impair membrane fluidity, ion homeostasis, signalling, and transport processes essential for growth and secondary metabolism.

3.2.2 Oxidative stress and antioxidant responses

Oxidative stress is among the most common responses to botanical pesticide exposure. Disruption of photosynthesis, respiration, and membrane integrity increases ROS production, including superoxide radicals (O^{2+}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). Excess ROS damages proteins, lipids, nucleic acids, and cellular membranes (Werrie*et al*., 2020).

Monoterpenes such as α-pinene and β-pinene strongly induce oxidative burst responses. Studies in wheat, *Pisum sativum* Lam., and *Cicer arietinum* L. reported increased H_2O_2 accumulation, lipid peroxidation, electrolyte leakage, and malondialdehyde (MDA) levels following α-pinene exposure (Singh *et al*., 2018). β-pinene also increased lipoxygenase (LOX) activity and membrane injury despite elevated antioxidant enzyme activity (Chowhan *et al*., 2013). Similar ROS-mediated effects were reported for citronellol and essential oils from *Pogostemon benghalensis* (Burm.f.) Kuntze, *Monarda didyma* L., and *A. scoparia* (Werrie *et al*., 2020).

Plants counter oxidative injury by activating antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase. Increased activity of these enzymes reflects attempts to restore redox balance. However, prolonged oxidative stress eventually overwhelms antioxidant capacity, leading to lipid peroxidation, membrane leakage, and programmed cell death (Werrie*et al*., 2020).

MDAis a common biomarker of botanical-induced oxidative injury. Elevated MDA levels correlate with membrane deterioration, chloroplast dysfunction, and loss of cellular viability following exposure to carvacrol, citronellol, and monoterpene-rich essential oils. ROS signalling also interacts with phytohormones, calcium signalling, and transcriptional regulators, amplifying downstream phytotoxic responses (Werrie*et al*., 2020).

3.2.3 Hormonal and signalling perturbation

Botanical pesticides significantly alter phytohormonal balance and intracellular signalling. Many monoterpenes disrupt auxin transport, ethylene biosynthesis, jasmonic acid signalling, and calcium-mediated signal transduction, thereby affecting plant growth and development.

Citral rapidly inhibits polar auxin transport and disrupts microtubule organisation in *Arabidopsis* roots, causing abnormal root morphology, impaired cell division, and altered meristem structure (Graña *et al*., 2013). Farnesene similarly affects PIN-FORMED (PIN) auxin transport proteins, resulting in reduced gravitropism and altered root architecture (Araniti *et al*., 2016). Increased ethylene production frequently accompanies these effects and contributes to chlorosis, senescence, and growth inhibition (Werrie *et al*., 2020).

Monoterpene-induced membrane depolarisation also triggers Ca^{2+} influx, activating stress and defence signalling pathways. ROS generated during oxidative burst interact with mitogen-activated protein kinases (MAPKs), transcription factors, and hormonal pathways, linking stress signalling with metabolic regulation (Marcec *et al*., 2019). Jasmonic acid signalling is particularly important because it regulates alkaloid and terpenoid biosynthesis. Exposure to myrcene and ocimene activated jasmonate-responsive genes and octadecanoid pathway metabolism in *Arabidopsis thaliana* (L.) Heynh (Werrie *et al*., 2020).

At low concentrations, some botanical compounds induce defence priming rather than toxicity. Mint volatiles enhanced defence gene expression in soybean through histone acetylation-mediated priming, while thyme oil induced pathogenesis-related (PR) genes in apple against *Botrytis cinerea* Pers. (Werrie *et al*., 2020). These signalling pathways may also contribute to defence priming responses discussed later.

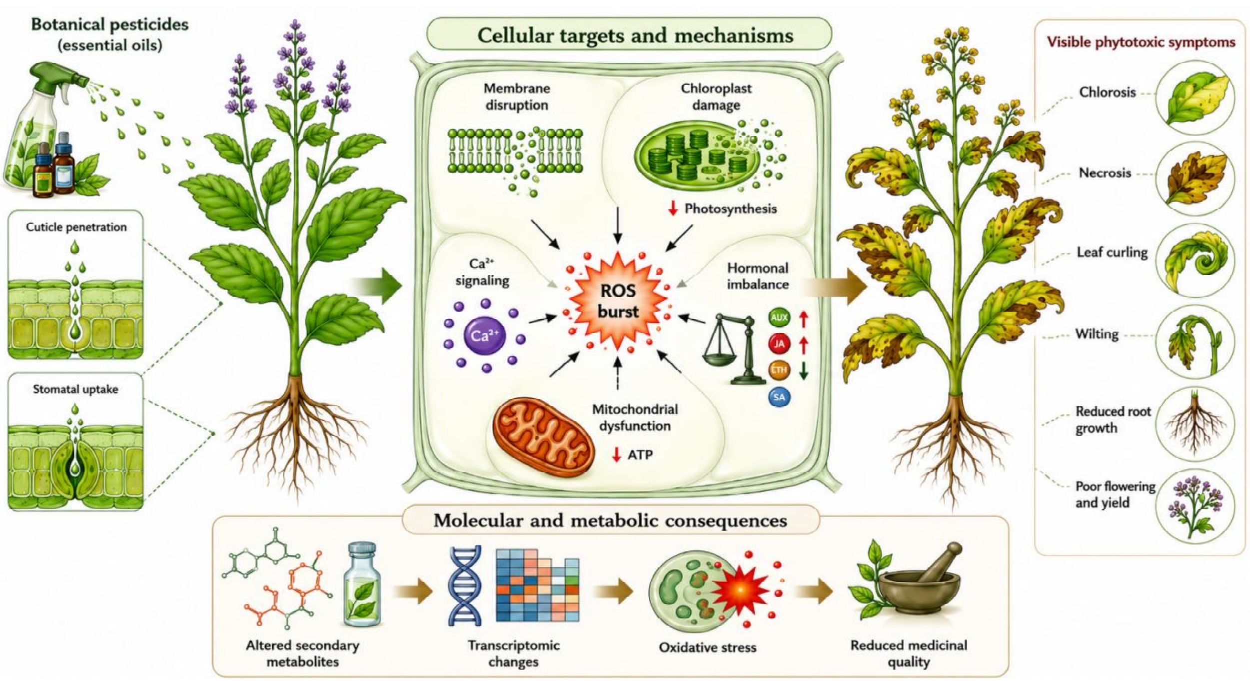
3.2.4 Omics insights into phytotoxicity

Transcriptomics, proteomics, and metabolomics have greatly improved the understanding of botanical pesticide phytotoxicity. Omics approaches reveal coordinated metabolic and regulatory responses that are not detectable through conventional physiological assays(Varadharajan *et al*., 2025).

Transcriptomic studies show that volatile monoterpenes induce large-scale expression of stress-responsive genes, detoxification enzymes, defence transcription factors, and hormone signalling components (Riedlmeier *et al*., 2017). Exposure to myrcene and ocimene activated jasmonate-associated pathways and induced numerous stress-related transcripts in *A. thaliana* (Werrie *et al*., 2020). Similar responses occurred after citral and monoterpene exposure, including altered expression of genes related to cytoskeleton organisation, oxidative stress regulation, and membrane stability.

Metabolomic studies demonstrate extensive metabolic disturbances beyond visible injury. Exposure to *Origanum vulgare* L. and other essential oils altered amino acid pools, carbohydrate metabolism, organic acid profiles, and phenylpropanoid intermediates. Disruption of glutamate and aspartate metabolism was associated with chlorosis and necrosis due to glutamine synthetase inhibition and ammonia accumulation, which impaired photosystem activity (Araniti *et al*., 2017).

Proteomic analyses further identified changes in antioxidant proteins, respiratory enzymes, membrane-associated proteins, and cytoskeletal components (Yan *et al.,* 2022). Collectively, omics studies show that botanical phytotoxicity involves interconnected oxidative, hormonal, metabolic, and structural disturbances affecting plant physiology and medicinal quality.



Source : Made by us using Canva AI software

Figure 1: Cellular targets, oxidative stress mechanisms, and visible phytotoxic symptoms induced by botanical pesticides in medicinal plants, illustrating their effects on photosynthesis, membrane integrity, ROS generation, and secondary metabolism.

3.2.5 Dose-dependent phytotoxicity and exposure scenarios

Phytotoxicity from botanical pesticides is highly dose-dependent and varies with compound type, crop species, and environmental conditions. Essential oil formulations commonly induce chlorosis, necrosis, membrane injury, and growth suppression at concentrations above 0.5-1.0% v/v in sensitive MAPs (Bakkali *et al*., 2008; Isman, 2020). Monoterpenes such as citral, β-pinene, and citronellol also reduce chlorophyll content, photosynthetic efficiency, and membrane stability in a concentration-dependent manner (Werrie *et al*., 2020).

Repeated applications in greenhouse MAP production may cause cumulative oxidative stress and metabolic disruption even when individual treatments appear safe. Phytotoxicity risk further increases during the flowering and reproductive stages.

Optimised formulations, however, can provide effective pest control with minimal crop injury. Essential oil from *C. angustifolia* leaves showed strong insecticidal and repellent activity against stored-grain pests without affecting paddy seed germination or seedling growth (Paul *et al*., 2024).

3.2.6 Medicinal crop-specific sensitivity

Sensitivity to botanical phytotoxicity varies among medicinal plants due to differences in cuticle composition, membrane properties, antioxidant capacity, and secondary metabolism. According to the conceptual model depicted in Figure 1, excessive exposure to botanical pesticides may impair photosynthesis and cellular homeostasis through ROS-mediated toxicity in different medicinal plants. Lamiaceae species such as *Mentha*, *Ocimum*, and *Thymus* are generally more sensitive to essential oil phytotoxicity because of efficient terpene uptake systems (Bakkali *et al*., 2008). Apiaceae crops show moderate sensitivity, whereas solanaceous MAPs such as *Withania* and *Datura* exhibit relatively greater tolerance to alkaloid-based botanicals.

Phytotoxicity also depends on botanical source, extraction method, concentration, and application frequency. Citrus-derived limonene formulations caused severe foliar injury in *Gardenia* spp. and ginger (*Zingiber officinale* Roscoe) (Hollingsworth, 2005). Neem (*A. indica*) formulations produced concentration-dependent phytotoxicity in several crops. Oily neem extracts caused leaf lesions in *Ixora coccinea* L., whereas higher neem oil concentrations induced scorching, growth retardation, reduced yield, uneven grain ripening, and plant mortality in cabbage (*B. oleracea capitata*), mustard (*B. juncea*), and plum (*Prunus domestica* L.) after repeated applications (Esparza-Díaz *et al*., 2010; Loke *et al*., 1992; Bajwa and Zimmermann, 1997).

In greenhouse-grown herbs such as lavender (*Lavandula stoechas* L.), oregano (*O. vulgare*), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), and St. John's wort (*Hypericum perforatum* L.), botanical insecticides containing pyrethrin, cinnamaldehyde, and potassium salts of fatty acids caused visible foliar injury, although later regrowth partially compensated for damage (Cloyd and Cycholl, 2002).

Oil composition further influences phytotoxicity. Oxygenated monoterpenes and phenylpropanoids generally exhibit stronger membrane-disruptive effects than sesquiterpenes (Werrie *et al*., 2020). In contrast, *C. angustifolia* leaf essential oil showed strong insecticidal activity without impairing paddy seed germination or seedling growth, indicating selective crop safety (Paul *et al*., 2024). These findings highlight the need for crop-specific phytotoxicity evaluation before large-scale botanical pesticide application.

4. Alterations in secondary metabolites and medicinal quality

4.1 Qualitative shifts in metabolite profiles

Secondary metabolite composition in MAPs is highly sensitive to botanical pesticides and essential oil formulations. Alterations in terpenoids, phenylpropanoids, alkaloids, and other bioactives can directly affect medicinal quality, pharmacological activity, and pharmacopeial compliance. Unlike synthetic pesticides, botanical pesticides contain volatile organic compounds (VOCs) that interact with cellular metabolism, membranes, enzymes, and phytohormonal signalling, thereby modifying metabolite biosynthesis and accumulation. Essential oil constituents penetrate plant tissues through stomata and cuticular layers and interact with intracellular targets. These compounds disrupt membrane organisation, ion fluxes, ROS signalling, and defence pathways (Werrie *et al*., 2020), thereby altering terpene, phenylpropanoid, and alkaloid biosynthesis.

Botanical pesticide-induced oxidative and membrane-associated stress responses described earlier may substantially alter secondary metabolite biosynthesis and chemotype composition in MAPs. Compounds such as α-pinene, β-pinene, citronellol, and *A. scoparia* oil generate ROS and alter physiological and metabolic processes (Abrahim *et al*., 2000; Werrie *et al*., 2020). Monoterpenes, including myrcene and ocimene, also induce defence-related gene expression (Godard *et al*., 2008). Essential oils further modify membrane lipids and sterol composition, thereby affecting metabolite compartmentalisation and chemotype (Bakkali *et al*., 2008; Werrie *et al*., 2020). Plants detoxify exogenous VOCs through oxidation, reduction, glycosylation, and conjugation pathways, indirectly altering endogenous metabolite pools. These qualitative changes often escape detection by conventional single-marker quality-control methods, highlighting the importance of metabolomic, proteomic, and transcriptomic approaches for MAP quality assessment (Wolfender *et al*., 2015).

4.2 Phytotoxicity versus elicitation/priming

Botanical pesticide-induced elicitation has emerged as a dual-benefit strategy in MAP cultivation, where mild stress stimulates secondary metabolism through jasmonate, salicylate, or ethylene signalling (Banani *et al*., 2018). At low concentrations, several botanical actives mimic biotic stress and activate defence-related transcription factors and biosynthetic enzymes. Essential oils exhibit strong dose-dependent duality, acting as defence elicitors at low doses but phytotoxic agents at higher concentrations (Souri *et al*., 2019). This hormetic response involves activation of interconnected stress and defence signalling pathways (Massimo, 2012). Volatiles such as myrcene and ocimene induced methyl jasmonate-like defence responses and activated octadecanoid pathways (Godard *et al*., 2008).

Priming effects have also been reported in crops. Mint volatiles enhanced transcription of defence genes in soybean (Sukegawa *et al*., 2018). Thyme oil induced PR genes in apple (Banani *et al*., 2018) and increased phenolics and peroxidase activity in tomato (Ben-Jabeur *et al*., 2015). Cinnamon oil, eugenol, and citronellal formulations also induced SAR-related enzymes, including β-1,3-glucanase, chitinase, and peroxidase (Perina *et al*., 2019; Lucas *et al*., 2012). However, many studies lack proper dose-response analysis and clear differentiation between elicitation and biomass reduction caused by phytotoxicity.

The same essential oil may be beneficial at low concentrations but toxic at higher doses. Responses vary with application method, exposure duration, crop stage, and environmental conditions (Werrie *et al*., 2020). Even low concentrations may induce membrane disruption, oxidative stress, photosynthetic inhibition, mitochondrial dysfunction, and microtubule disorganisation (Bakkali *et al*., 2008). The narrow threshold between elicitation and toxicity creates uncertainty in MAP production. Currently, no standard regulatory framework defines safe elicitor doses for medicinal crops.

4.3 Medicinal quality deterioration versus enhancement

The effects of botanical pesticides on medicinal quality depend on pharmacopoeial standards, EMA/HMPC (European Medicines Agency; Committee on Herbal Medicinal Products) guidelines, residue regulations, and export requirements. Botanical pesticides may either enhance or reduce medicinal quality depending on concentration, crop species, formulation, timing, and environmental conditions. Low-dose exposure may stimulate defence-associated biosynthesis and increase valuable terpenoids, phenolics, or alkaloids. In contrast, excessive exposure may suppress biosynthetic pathways, induce oxidative damage, reduce biomass, and alter chemotype composition (Isah, 2019).The medicinal quality effects of botanical pesticides are closely linked with the oxidative and metabolic disturbances discussed in Section 3.However, moderate oxidative signalling may activate salicylic acid- and jasmonic acid-mediated defence pathways, increasing phenolics, phytoalexins, terpenoids, and other defence metabolites (Werrie *et al*., 2020). Consequently, medicinal quality depends on the balance between defence activation and phytotoxic injury.

Many botanical pesticide-induced quality defects remain visually undetectable because phytotoxicity assessments often focus only on external injury symptoms (Dayan *et al*., 2009). Subtle changes in chemotype composition, metabolite ratios, or degradation products may still result in non-compliance with pharmacopoeial standards. Essential oil composition already varies with chemotype and environmental conditions, and additional metabolic variation induced by botanical pesticides may further compromise raw material consistency. Therefore, metabolomic, transcriptomic, and proteomic approaches are increasingly important for detecting phytochemical alterations and distinguishing beneficial elicitation from medicinal quality deterioration (Rai *et al*., 2017)

5. Residue dynamics and ecotoxicological impacts of botanical pesticides in medicinal agroecosystems

5.1 Residue persistence and transformation in medicinal plant products

Residue persistence in processed medicinal products is an important concern because dried herbal materials possess low water activity and limited microbial degradation, which can prolong the stability of botanical pesticide residues. As a result, residues may persist during storage, processing, and pharmaceutical extraction, creating quality-control and consumer exposure concerns within herbal supply chains. In addition to parent compounds, degradation metabolites remain poorly characterised despite their toxicological and ecological significance, as several metabolites may retain equal or even greater biological activity than the original compounds. For example, rotenone degradation produces rotenolone and related metabolites that remain ichthyotoxic and insecticidal (Ling, 2003), while nicotine degradation generates nornicotine, cotinine, and myosmine, which retain nicotinic acetylcholine receptor (nAChR) activity. Pereira *et al*. (2025) reported that trans-cinnamaldehyde undergoes oxidation, aldehyde reduction, epoxidation, and dihydroxylation, whereas the degradation pathways of carvacrol vary depending on the microbial taxa involved. These findings highlight the need for comprehensive residue assessments that consider both parent botanical compounds and their transformation products to ensure the safety, quality, and regulatory compliance of medicinal plant products.

5.2 Aquatic fate and environmental ecotoxicology

Runoff, leaching, and spray drift may transport botanical pesticide residues into aquatic ecosystems. Despite their reputation as safer alternatives, several botanical compounds show strong aquatic toxicity. Rotenone is particularly hazardous because it inhibits mitochondrial electron transport and is highly toxic to fish and aquatic invertebrates even at low concentrations (Ling, 2003). Pereira *et al*. (2025) further reported that rotenone and rotenolone persistence varies with soil and environmental conditions, increasing contamination risks after runoff events.

Pyrethrins and pyrethrum extracts also pose significant aquatic risks. Although rapidly degraded by sunlight, they remain highly toxic to fish and aquatic arthropods because they disrupt sodium channel regulation in nervous tissues (Casida and Quistad, 1995). Several monoterpene-rich essential oil constituents are generally less persistent due to volatilisation and oxidation. However, transient exposure may still disrupt freshwater invertebrates, microbial communities, and fish olfactory systems at ecologically relevant concentrations (Bakkali *et al*., 2008). In addition, degradation products formed through oxidation or hydrolysis may remain biologically active. The lack of toxicity data for most botanical metabolites remains a major limitation in environmental risk assessment.

5.3 Non-targetorganisms and multi-trophic ecological effects

5.3.1 Beneficial arthropods and natural enemies

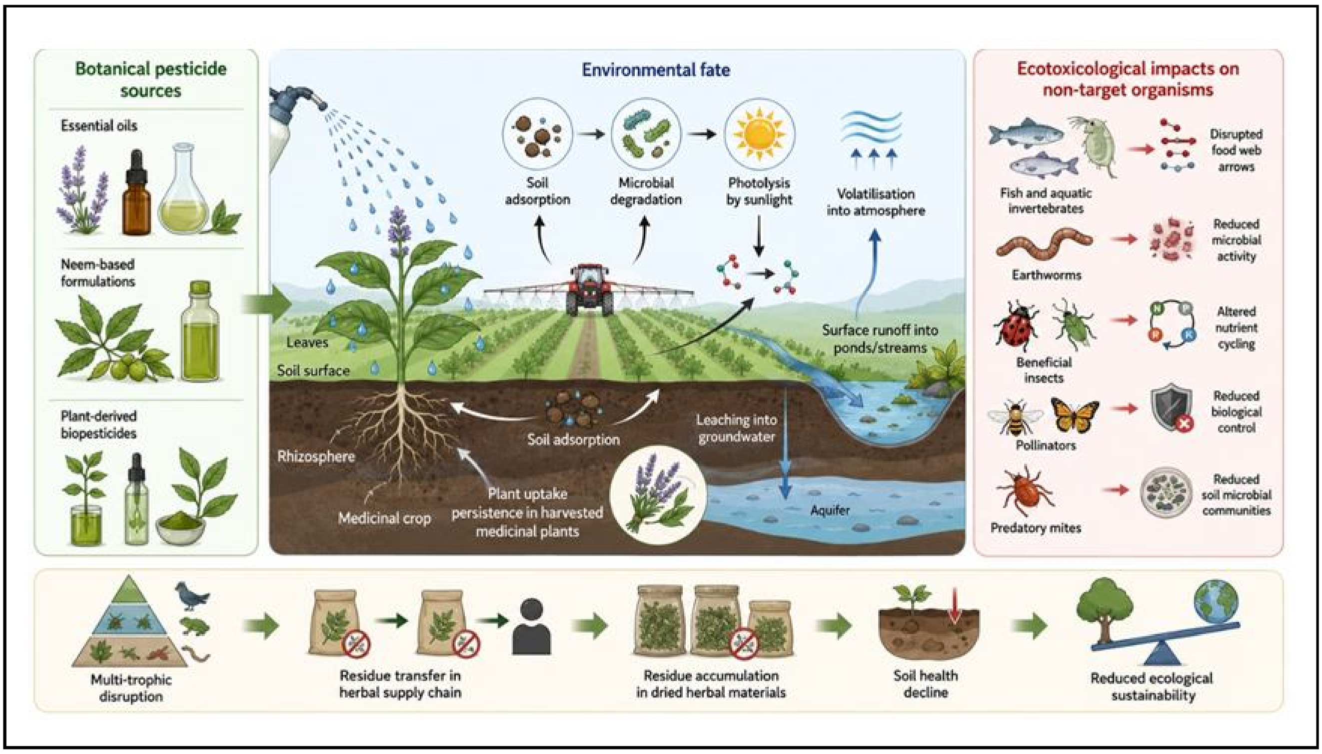
Botanical pesticides may negatively affect beneficial arthropods involved in biological control. Pyrethrin and azadirachtin formulations exhibited acute toxicity toward predators such as *Chrysoperl acarnea* Stephens.,*Coccinella septempunctata* L., and *Orius insidiosus* Say., with mortality comparable to that of some synthetic pyrethroids (Corcos, 2020). As shown in Table 2, essential oil-based botanical pesticides can exert varying ecotoxicological effects on non-target fauna and microbial communities. Products derived from *Melia azedarach* L. and *A. indica* also affected the parasitoid *Cotesia plutellae* Haliday (Charleston *et al*., 2005). Essential oils rich in thymol and carvacrol were toxic to parasitoids such as *Trichogramma* spp. and *Aphidius colemani*, important natural enemies in greenhouse MAP systems. Pereira et *al*. (2025) reported significant mortality of *Propylea quatuordecimpunctata* L. larvae after pyrethrin exposure, whereas citrus and thyme oils produced species-specific effects.

Table 2: Ecotoxicological effects of essential oil-based botanical pesticides on non-target organisms relevant to MAP agroecosystems

Botanical pesticide (EO)	Non-target organism	Effect level	Key effect	LC_{50}/LD_{50} or EC_{50}	Comparative synthetic pesticide impact	References
Mentha pulegium EO	*Chrysoperlacarnea* (green lacewing)	Lethal + sublethal	Reduced egg hatching rate; toxicity to eggs	Not reported	Not reported	Azimi Zadeh and Ahmadi, 2018
Rosmarinus officinalis EO	*Chrysoperlacarnea* (green lacewing)	Sublethal	Negative effects on egg hatching; low larval mortality	Not reported	Not reported	Azimi Zadeh and Ahmadi, 2018
Ocimum basilicum EO	*Habrobraconhebetor* (parasitoid wasp)	Lethal + sublethal	Mortality and altered life-history traits	LC_{50}= 0.84 ml L^{-1}	Not reported	Ahmadpour *et al*., 2021
Melissa officinalis EO	*Neoseiulusbarkeri*, *Typhlodromipsswirskii* (predatory mites)	Lethal + sublethal	Mortality, reduced food consumption and fecundity	Not reported	Organic solvent formulation mitigated adverse effects	Abdel Kader *et al*., 2015

Cymbopogon citratus EO	*Podisusnigrispinus* (predatory bug)	Sublethal	Reduced respiratory and locomotor activity	Not reported	Not reported	Brügger *et al.*, 2019
Coriandrum sativum EO	*Cyrtorhinus lividipennis* (predatory mirid bug)	Sublethal/ behavioural	Attraction and orientation responses	Not reported	Not reported	Liu *et al.*, 2019
Citrus EO-based formulation (Prev-am ®)	*Nesidiocoris tenuis* (predatory mirid bug)	Mild sublethal	Increased predatory voracity and locomotor activity	Not reported	Lambda-cyhalothrin reduced predatory activity more strongly	Soares *et al.*, 2019
Origanum vulgare EO	*Trichospilus pupivorus* (parasitoid wasp)	Mild lethal	Lower toxicity toward parasitoid adults compared with the target pest	LC_{50} = 2.79%	More selective than against *Diaphania hyalinata*	Moreira da Silva *et al.*, 2020
L. angustifolia EO constituents	*C. glomerata* (parasitoid wasp)	Mild lethal	Lower toxicity than conventional insecticides	LC_{50} = 0.01 mg cm^{-3}	Approximately 1,430 times less toxic than dichlorvos	Yi *et al.*, 2016
Citrus sinensis cv. "Pera" EO	*Typhlodromus ornatus* (predatory mite)	Negligible/ sublethal	No significant effect on population growth	Not reported	Not reported	Brito *et al.*, 2021

Abbreviations: EO; Essential oil, LC_{50}; Lethal concentration, LD_{50}; Lethal dose, EC; Effective concentration



Source: Made by us using Canva AI software

Figure 2: Environmental fate, residue movement, and ecotoxicological impacts of botanical pesticides MAP agroecosystems, including degradation pathways, non-target effects, and implications for ecological sustainability.

Predatory mites of the family Phytoseiidae are important in medicinal crop IPM systems. Paspati *et al.* (2023) reported low to moderate mortality of *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius swirskii* Athias-Henriot after exposure to extracts of *Chenopodium ambrosioides* (L.) Mosyakin and Clemants, *C. aurantium*, and olive oil-based formulations. These findings challenge the assumption that botanical pesticides are universally safe for biological control agents.

5.3.2 Soil invertebrates and microbial communities

Among soil invertebrates, earthworms are the most widely studied ecotoxicological indicators. Formulations based on *Piper betle*, *Melaleuca cajuputi*, and *O. sanctum* generally showed low toxicity toward *Eisenia fetida* and *Eudrilus eugeniae* under experimental conditions (Pereira *et al.*, 2025). However, long-term effects on soil biodiversity remain poorly understood.

5.3.3 Multitrophic interaction networks in medicinal agroecosystems

Botanical pesticides may disrupt complex multi-trophic interactions within medicinal agroecosystems. They can alter herbivore-induced plant volatile (HIPV) emissions and interfere with chemical signalling involved in predator and parasitoid recruitment. Changes in plant secondary metabolism may therefore affect tritrophic communication among plants, herbivores, and natural enemies, potentially weakening biological control. The conceptual framework presented in Figure 2 demonstrates how botanical pesticide residues can affect soil health, aquatic systems, pollinators, and biological control agents within MAP agroecosystems

Soil-mediated interactions may also be disrupted through changes in microbial communities, nutrient cycling, and rhizosphere signalling. Pereira *et al*. (2025) reported that botanical pesticide accumulation may simultaneously affect microorganisms, arthropods, and soil enzymes, creating cascading ecological effects across trophic levels. However, these interactions remain poorly studied in medicinal crop systems, particularly under long-term field conditions. Future research should therefore integrate residue dynamics, microbial ecology, plant metabolomics, and trophic network analyses to better evaluate the sustainability of botanical pesticides in phytomedicine production systems.

6. Human health and toxicological risk assessment

6.1 Intrinsic toxicity of major botanical actives

Beyond phytotoxicological and ecological impacts, botanical pesticide residues may also pose occupational and consumer health concerns. Several botanical compounds nevertheless exhibit important mammalian toxicological effects. Rotenone is one of the most studied botanical toxicants because it inhibits mitochondrial complex I, disrupts oxidative phosphorylation, and induces oxidative stress in neuronal tissues. Chronic exposure has been linked with dopaminergic neurodegeneration and Parkinsonian symptoms (Tanner *et al*., 2011).

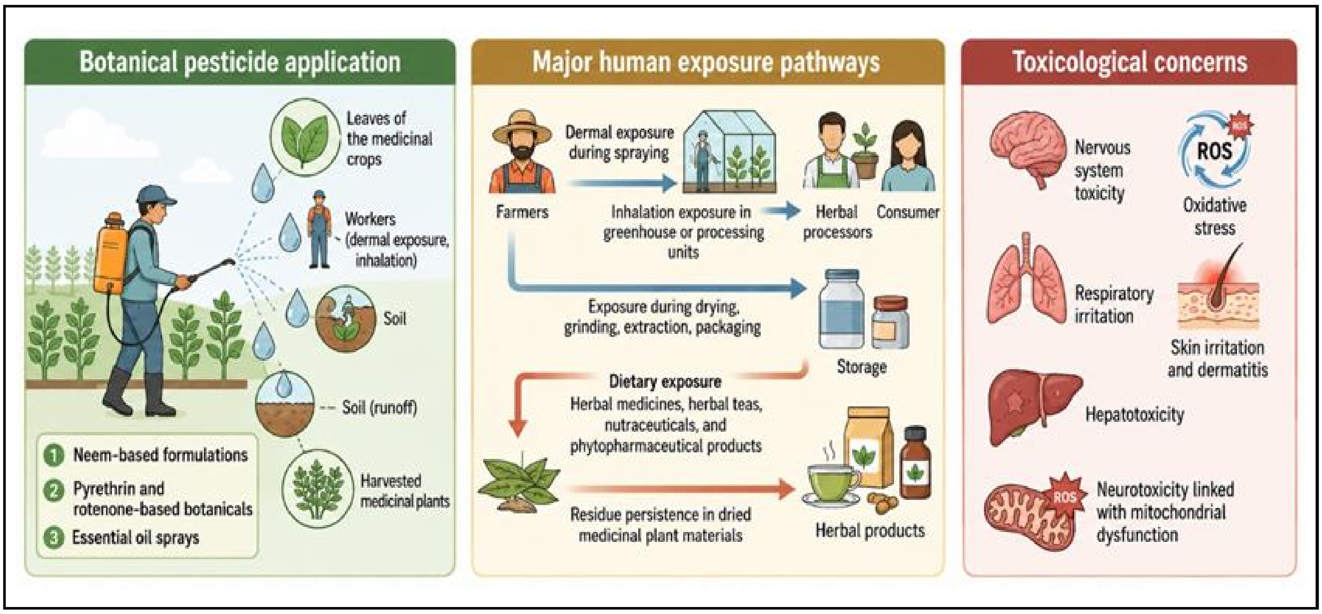
Pyrethrins generally show lower mammalian toxicity than many synthetic insecticides. However, occupational exposure may cause allergic dermatitis, respiratory irritation, and asthma-like symptoms in agricultural workers (Casida and Quistad, 1995). Nicotine-based formulations are highly toxic because nicotine strongly affects nAChRs. Dermal or inhalation exposure during handling may therefore cause severe poisoning.

Essential oil constituents such as thymol, eugenol, carvacrol, and other monoterpenes may also induce mucosal irritation, hepatotoxicity, and cytotoxic effects at high concentrations or after prolonged exposure (Bakkali *et al*., 2008). These findings demonstrate that botanical origin does not guarantee toxicological safety. Consequently, toxicodynamic evaluation remains essential before large-scale agricultural use.

6.2 Exposure scenarios: Growers, processors, and consumers

Human exposure to botanical pesticide residues inMAP systems occurs through occupational, processing, and dietary pathways. Farm workers are mainly exposed during mixing, spraying, harvesting, and post-application handling, particularly in greenhouses where aerosol persistence may increase inhalation risk (Damalas and Koutroubas, 2016).

Workers involved in drying, grinding, and extraction may also encounter concentrated residues and volatile compounds during processing. Consumer exposure primarily occurs through herbal medicines, herbal teas, nutraceuticals, and phytopharmaceutical products containing residual botanical compounds after harvest and storage. As illustrated in Figure 3, exposure to botanical pesticides may induce multiple toxicological responses such as oxidative stress, respiratory complications, liver toxicity, neurotoxicity, and skin irritation.



Source: Made by us using Canva AI software

Figure 3: Major human exposure pathways and potential toxicological effects associated with botanical pesticide residues in medicinal plant production and herbal products.

Persistent residues in stored herbal materials may contribute to prolonged consumer exposure (Booker *et al*., 2016). Repeated consumption of herbal preparations may therefore result in cumulative low-dose exposure to biologically active residues whose toxicological effects remain poorly characterised.

6.3 Toxicological risk assessment frameworks and existing gaps

Risk assessment frameworks for botanical pesticide residues remain poorly developed compared with those for synthetic pesticides. A major limitation is the lack of validated analytical methods and certified reference standards for many botanical compounds and their metabolites (Isman, 2006). Toxicological data are also incomplete, particularly for chronic toxicity, carcinogenicity, reproductive toxicity, endocrine disruption, and long-term dietary exposure.

Establishing acceptable daily intake (ADI) values is especially challenging because many botanical compounds naturally occur in medicinal plants and are also used as pesticides. This dual role complicates residue interpretation and exposure assessment. In addition, consumption data for HMPs are limited, despite evidence that frequent users may experience relatively high exposure levels (Jordan *et al.,* 2010). As a result, current risk assessments often rely on extrapolated data, creating major uncertainty in human health evaluation.

6.4 Regulatory perspectives and export standards

Regulations governing botanical pesticide residues in medicinal plants remain fragmented across international jurisdictions. Most residue standards are designed for food crops rather than medicinal plants, resulting in poor harmonisation for herbal products (European Medicines Agency, 2008).

The EU enforces strict contaminant and quality requirements for herbal medicines through the European Pharmacopoeia and EMA/HMPC guidelines. As shown in Table 3, different regulatory agencies have established varying standards for botanical pesticide residues in medicinal plants and herbal products. However, specific regulations for botanical pesticide residues remain limited. In the United States, several botanical pesticides are exempt from tolerance requirements under specific EPA conditions, although toxicologically important compounds such as rotenone remain regulated (USEPA, 2007).

Table 3: Regulatory frameworks for botanical pesticide residues in medicinal plant products

Regulatory framework	Jurisdiction	Key substance	MRL/guideline value	Regulated matrix	Notes	References
Regulation (EC) No. 396/2005	European Union	Azadirachtin	Default MRL generally 0.01 mg/kg, where no specific value is established	Herbal drugs, fresh herbs	No harmonised specific MRLs for most medicinal plants	European Commission, 2005
Regulation (EC) No. 396/2005	European Union	Pyrethrins	Commodity-specific MRLs generally range from 1-10 mg/kg, depending on the crop	Dried herbs, fresh produce	Natural pyrethrins are included under EU pesticide residue legislation; synergists are separately regulated	European Commission, 2005
Codex Alimentarius (CAC/MRL)	Global (FAO/WHO)	Pyrethrin I + II	Codex MRLs vary by commodity, commonly 0.5-10 mg/kg	Tea, dried herbs	Codex guidance is used internationally for trade and residue harmonisation	Codex Alimentarius Commission, 1993
Food Safety and Standards Regulations (FSSR), 2011	India	Botanical pesticides	No dedicated MRLs specified for most medicinal plants	Spices, herbs	Medicinal plants are often regulated under general food safety provisions	FSSAI, 2011
FIFRA / USEPA	United States	Rotenone	Restricted use; ecological hazard warnings mandatory	Environmental exposure matrices	Rotenone products require aquatic toxicity hazard statements	USEPA, 2007
Regulation (EC) No. 1107/2009	European Union	Quassia extract	Approved as a basic substance	Organic crop production systems	Limited residue data available for medicinal crops	European Commission, 2009
EMA/HMPC Guidelines	European Union	Pesticide residues in HMPs.	Generally follows limits analogous to the European Pharmacopoeia and toxic element guidance	HMPs	Residue quality assessment integrated into HMPC quality requirements	European Medicines Agency, 2013
WHO Guidelines	Global	Pesticide residues in medicinal plants	WHO recommendations based on quality and safety assessment frameworks	Dried medicinal plant material	Widely used reference framework for herbal medicine quality control	WHO, 2007

Abbreviations: EC; European Commission; MRL; Maximum residue rimit**,** EU; European Union, CAC, Codex Alimentarius Commission, FAO; Food and Agriculture Organisation,WHO;World Health Organisation, FIFRA; Federal Insecticide, Fungicide, and Rodenticide Act, USEPA; United States Environmental Protection Agency, EMA; European Medicines Agency, HMPC; Committee on Herbal Medicinal Products, HMPs; Herbal medicinal products.

Export-oriented medicinal plant industries, therefore, face major compliance challenges due to inconsistent residue standards, lack of harmonised maximum residue limits (MRLs), and limited monitoring protocols for phytomedicinal commodities. Strengthening residue surveillance, harmonising international regulations, and generating comprehensive toxicological datasets are essential for safe integration of botanical pesticides into medicinal crop systems.

7. Analytical methods, omics, and AI for phytotoxicology and residue analysis

7.1 Residue analysis and environmental monitoring methods

Residue analysis of botanical pesticides in MAP systems is highly challenging because herbal matrices contain abundant alkaloids, terpenoids, flavonoids, phenolics, and volatile oils that interfere with extraction, chromatographic separation, and ionisation processes (Pereira *et al*., 2025). Consequently, conventional multiresidue methods such as QuEChERS(Quick, Easy, Cheap, Effective, Rugged, and Safe) coupled with GC-MS/MS or LC-MS/MS often show limited sensitivity and selectivity for botanical residues. Matrix suppression and co-elution are particularly problematic for chemically unstable compounds such as azadirachtin, whosemultiple stereocentres complicate quantification (Grimalt *et al*., 2011). Matrix-matched calibration and isotopically labelled internal standards are therefore increasingly required.

Essential oil-derived residues present additional analytical difficulties because compounds such as thymol, carvacrol, eugenol, and limonene naturally occur in many medicinal plants. This complicates differentiation between endogenous metabolites and externally applied residues. Advanced platforms, including GC×GC-MS, HRMS, and UHPLC-MS/MS, are increasingly used to improve selectivity and trace-level detection (Pereira *et al*., 2025). Pereira *et al*. (2025) also highlighted the absence of standardised analytical protocols for monitoring botanical pesticides in soils and herbal matrices.

Environmental monitoring of botanical pesticides in soil, water, and air remains limited compared with synthetic pesticides. Residue studies require highly sensitive analytical methods because botanical compounds often occur at low concentrations and degrade rapidly (Pereira *et al*., 2025). Emerging technologies such as biosensors, immunoassays, surface-enhanced Raman spectroscopy (SERS), and portable spectrometric devices are increasingly explored for rapid field-based monitoring in medicinal agroecosystems.

7.2 Metabolomics and other omics in phytotoxicology

Advanced omics platforms are increasingly applied for residue-associated phytotoxicological assessment in MAP systems. Untargeted metabolomics, particularly nuclear magnetic resonance (NMR) and LC-MS-based approaches, enables a comprehensive assessment of biochemical changes induced by botanical exposure (Wolfender *et al*., 2015; Emwas *et al*., 2019). These methods reveal alterations in amino acid metabolism, phenylpropanoid biosynthesis, carbohydrate fluxes, antioxidant pathways, and secondary metabolism that are often undetectable by targeted analyses. Citrus-derived limonene formulations caused severe phytotoxicity in *G.augusta* and ginger (*Z. officinale*), producing foliar injury and tissue damage (Hollingsworth, 2005).

Transcriptomic tools such as RNA sequencing (RNA-Seq) and microarrays provide insight into molecular stress responses induced by botanical pesticides. Exposure alters expression of genes associated with photosynthesis inhibition, oxidative stress defence, xenobiotic detoxification, cytochrome P450 monooxygenases, glutathione-S-transferases (GSTs), and ATP-binding cassette (ABC) transporters (Werrie *et al*., 2020). These responses indicate activation of complex defence and detoxification pathways in medicinal plants.

Proteomics remains less explored but offers strong potential for identifying enzyme targets, stress proteins, and post-translational modifications associated with phytotoxicity. Integration of metabolomics, transcriptomics, and proteomics through systems biology approaches is increasingly important for understanding the complex physiological effects of botanical pesticides. Combined omics datasets may also help identify early stress biomarkers and support predictive models for residue-associated quality deterioration in medicinal crops.

7.3 AI-assisted toxicity and fate prediction

Artificial intelligenceand machine learning are emerging as important tools for predicting botanical pesticide toxicity, persistence, and phytotoxicity. Quantitative structure-activity relationship (QSAR) models estimate toxicity and environmental behaviour from molecular structure and physicochemical properties (Isangadighi *et al*., 2024). These approaches can accelerate hazard identification when experimental data are limited. However, the lack of standardised phytotoxicity datasets remains a major limitation for robust AI model development.

Deep learning approaches combined with metabolomics and hyperspectral imaging are also being explored for early detection of phytotoxic stress in medicinal crops. Convolutional neural networks (CNNs) integrated with hyperspectral imaging can detect subtle biochemical and physiological changes before visible symptoms appear, enabling non-destructive real-time monitoring (Singh *et al*., 2018). AI-assisted interpretation of LC-MS and GC-MS metabolomic fingerprints may further improve discrimination between stressed and non-stressed plants.

Molecular docking and AI-driven molecular dynamics analyses are additionally used to predict interactions between botanical compounds and plant enzymatic targets, providing mechanistic insight into phytotoxicity (Roy *et al.,* 2016). AI-assisted environmental fate models can also predict degradation kinetics, metabolite formation, soil adsorption, and ecotoxicological risks under different agroecological conditions. Pereira *et al*. (2025) highlighted the growing importance of computational tools because laboratory-based environmental assessments are often expensive and time-consuming.

Overall, integration of AI, omics technologies, and advanced analytical chemistry is rapidly transforming botanical pesticide phytotoxicology and residue science. These approaches may improve residue monitoring, regulatory assessment, environmental risk evaluation, and medicinal crop quality management.

8. Future research priorities and emerging challenges in botanical pesticide phytotoxicology

Major knowledge gaps in botanical pesticide phytotoxicology require interdisciplinary research in MAPsystems. Recent advances (2021-2026) in nanoformulations, omics, AI-assisted prediction, and smart delivery systems have accelerated botanical pesticide development. Nanoencapsulated azadirachtin, thymol, eugenol, and carvacrol improve efficacy at lower doses but may increase phytotoxicity through enhanced cuticular penetration and cellular uptake (Kah *et al*., 2013; Sarmah *et al*., 2025). Future research should prioritise long-term, multi-site field studies under realistic cultivation conditions. Standardised multi-omics studies in *M. piperita*, *O.basilicum*, *C. roseus*, and *A. annua* are also needed to identify early phytotoxicity biomarkers.

Recent omics studies demonstrate the value of molecular diagnostics for detecting botanical pesticide stress. Transcriptomic analyses showed induction of MYB and WRKY transcription factors involved in phenylpropanoid and terpenoid metabolism, together with suppression of photosynthesis-related genes under stress (Rabeh *et al*., 2025). UHPLC-QTOF-MS metabolomics has also been used in *C. roseus* to characterise alkaloid profiles and treatment-induced metabolic variation in vincristine- and vinblastine-producing tissues (Pan *et al*., 2016). QSAR and machine learning models further show potential for predicting phytotoxicity of terpenoid and phenolic compounds. Graph neural network (GNN)-based models are also being explored to predict persistence, uptake, and transformation behaviour in soil systems (Anand *et al.,* 2024).

Despite these advances, major ecological and regulatory challenges remain unresolved. Most studies on EO-based pesticides focus on efficacy, whereas impacts on pollinators, natural enemies, and other non-target organisms remain poorly understood (Giunti *et al*., 2022). Future studies should therefore evaluate ecological safety, biological control compatibility, and multi-trophic interactions in integrated pest management systems. Hormetic and biphasic dose-response relationships also remain insufficiently studied despite increasing evidence of sub-NOEL stimulatory effects (Belz and Duke, 2022).

Emerging technologies such as nanoformulations, eco-compatible carriers, microwave-assisted extraction, and enzyme-assisted extraction also require ecotoxicological evaluation (Kah *et al*., 2013). Although botanical pesticide registration has been simplified in some regions (Isman, 2020), regulatory ambiguity and stricter ecological risk assessment continue to limit commercialisation, particularly in the EU (Vekemansand Marchand, 2020).

9. Conclusion

Botanical pesticides are increasingly promoted as sustainable alternatives to synthetic agrochemicals in MAP production systems because of their biodegradability, broad-spectrum bioactivity, and compatibility with organic and integrated pest management strategies. However, growing evidence demonstrates that these compounds are not universally safe. Depending on their chemical composition, concentration, formulation, and exposure conditions, botanical pesticides may induce significant phytotoxic, ecotoxicological, and toxicological effects. Current research indicates that botanical pesticides can disrupt membrane integrity, photosynthesis, mitochondrial function, redox homeostasis, hormonal signalling, and secondary metabolism, leading to visible crop injury as well as hidden biochemical and molecular perturbations. In MAP systems, such effects are particularly important because alterations in alkaloids, terpenoids, phenolics, and essential oils directly influence medicinal quality, pharmacological efficacy, and pharmacopeial compliance. Moreover, residue persistence, degradation metabolites, and impacts on non-target organisms across soil, aquatic, and multi-trophic ecosystems remain insufficiently understood.

Recent advances in analytical chemistry, omics technologies, nanoformulations, and AI-assisted prediction have substantially improved understanding of botanical pesticide behaviour, residue dynamics, and phytotoxic mechanisms. Nevertheless, major gaps persist regarding long-term ecological safety, chronic human exposure, standardised residue monitoring, and regulatory harmonisation for medicinal crops. Future research should therefore prioritise integrated multi-omics investigations, realistic field-based phytotoxicity assessments, ecological risk evaluation, and development of harmonised regulatory frameworks specific to phytomedicinal production systems.Overall, the sustainable use of botanical pesticides in MAP cultivation will depend on achieving an optimal balance between pesticidal efficacy, medicinal quality preservation, environmental safety, and human health protection.Future research should focus on developing standardised phytotoxicity assessment protocols, understanding long-term ecological impacts, and establishing harmonised residue monitoring frameworks for medicinal plant production systems

Acknowledgements

All the authors express their gratitude to the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Conflict of interest

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

References

Abdel Kader, M.M.; Momen, F.M.; Sammour, E.A.; Aly, S.M. and Fahim, S.F. (2015). Influence of *Melissa officinalis* essential oil and its formulation on *Typhlodromips swirskii* and *Neoseiulus barkeri* (Acari: Phytoseiidae). Acta Phytopathologica et Entomologica Hungarica, **50**:139-148.

Abrahim, D.; Braguini, W.L.; Kelmer-Bracht, A.M. and Ishii-Iwamoto, E.L. (2000). Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. Journal of Chemical Ecology, **26**:611-624.

Ahmadpour, R.; Rafiee-Dastjerdi, H.; Naseri, B.; Hassanpour, M.; Ebadollahi, A. and Mahdavi, V. (2021). Lethal and sublethal toxicity of some plant-derived essential oils in the ectoparasitoid wasp, *Habrobracon hebetor* Say (Hymenoptera: Braconidae). International Journal of Tropical Insect Science, **41**:601-610.

Anand, G., Koniusz, P., Kumar, A., Golding, L. A., Morgan, M. J., and Moghadam, P. (2024). Graph neural networks-enhanced relation prediction for ecotoxicology (GRAPE). Journal of Hazardous Materials, **472**: 134456.

Araniti, F.; Grana, E.; Krasuska, U.; Bogatek, R.; Reigosa, M.J.; Abenavoli, M.R. and Sánchez-Moreiras, A.M. (2016). Loss of gravitropism in farnesene-treated Arabidopsis is due to microtubule malformations related to hormonal and ROS imbalance. PLoS ONE, **11**:e0160202.

Araniti, F.; Sánchez-Moreiras, A.M.; Graña, E.; Reigosa, M.J. and Abenavoli, M.R. (2017). Terpenoid trans-caryophyllene inhibits weed germination and induces plant water status alteration and oxidative damage in adult Arabidopsis. Plant Biology, **19**:79-89.

Arumugam, T.; Premalashmi, V. and Theradimani, M. (2010). Effect of biopesticides, organic amendments and chemicals on the incidence of leaf spot (*Cercospora rauwolfia*) in sarpagandha. Green Farming, **1**:633-635.

Azimi Zadeh, N. and Ahmadi, K. (2018). The impact of several plant extracts and essential oils on pistachio psylla (*Agonoscena pistaciae*) and its natural enemy, *Chrysoperlacarnea*. Journal of Nuts, **9**:159-168.

Bainard, L.D.; Isman, M.B. and Upadhyaya, M.K. (2006). Phytotoxicity of clove oil and its primary constituent eugenol and the role of leaf epicuticular wax in the susceptibility to these essential oils. Weed Science, **54**:833-837.

Bajwa, G.A. and Zimmermann, G. (1997). Efficacy of *Azadirachta indica* A. Juss (neem) against larvae of *Lymantria dispar* Linn. (gypsy moth) in the laboratory: pp:23-28.

Bakkali, F.; Averbeck, S.; Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils: A review. Food and Chemical Toxicology, **46**(2):446-475.

Banani, H.; Olivieri, L.; Santoro, K.; Garibaldi, A.; Gullino, M.L. and Spadaro, D. (2018). Thyme and savoury essential oil efficacy and induction of resistance against *Botrytis cinerea* through priming of defence responses in apple. Foods, **7**:11.

Belz, R.G. and Duke, S.O. (2022). Modelling biphasic hormetic dose responses to predict sub-NOAEL effects using plant biology as an example. Current Opinion in Toxicology, **29**:36-42.

Ben-Jabeur, M.; Ghabri, E.; Myriam, M. and Hamada, W. (2015). Thyme essential oil as a defence inducer of tomato against grey mould and Fusarium wilt. Plant Physiology and Biochemistry, **94**:35-40.

Bernhoft, A. (2010). A brief review of bioactive compounds in plants. Bioactive Compounds in Plants-Benefits and Risks for Man and Animals, **50**:11-17.

Booker, A.; Johnston, D. and Heinrich, M. (2016). Value chains of herbal medicines: Research needs and key challenges in the context of ethnopharmacology. Journal of Ethnopharmacology, **140**(3):624-633.

Brito, D.R.B.; Pinto-Zevallos, D.M.; de Sena Filho, J.G.; Coelho, C.R.; Nogueira, P.C.L.; de Carvalho, H.W.L. and Teodoro, A.V. (2021). Bioactivity of the essential oil from sweet orange leaves against the coconut mite *Aceriaguer reronis* (Acari: Eriophyidae) and selectivity to a generalist predator. Crop Protection, **148**:105737.

Brügger, B.P.; Martínez, L.C.; Plata-Rueda, A.; Castro, B.M. de C. e.; Soares, M.A.; Wilcken, C.F.; Carvalho, A.G.; Serrão, J.E. and Zanuncio, J.C. (2019). Bioactivity of the *Cymbopogon citratus* (Poaceae) essential oil and its terpenoid constituents on the predatory bug, *Podisus nigrispinus* (Heteroptera: Pentatomidae). Scientific Reports, **9**:1-8.

Caboni, P.; Sherer, T.B.; Zhang, N.; Taylor, G.; Na, H.M.; Greenamyre, J.T. and Casida, J.E. (2004). Rotenone, deguelin, their metabolites, and the rat model of Parkinson's disease. Chemical Research in Toxicology, **17**(11):1540-1548.

Casida, J.E. (1980). Pyrethrum flowers and pyrethroid insecticides. Environmental Health Perspectives, **34**:189-202.

Casida, J.E. and Durkin, K.A. (2013). Neuroactive insecticides: Targets, selectivity, resistance, and secondary effects. Annual Review of Entomology, **58**:99-117.

Casida, J.E. and Quistad, G.B. (1995). Pyrethrum flowers: Production, Chemistry, Toxicology, and Uses.

Charleston, D.S.; Kfir, R.; Dicke, M. and Vet, L.E.M. (2005). Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth. Biological Control, **33**:131-142.

Chaudhary, S.; Kanwar, R.K.; Sehgal, A.; Cahill, D.M.; Barrow, C.J.; Sehgal, R. and Kanwar, J.R. (2017). Progress on *Azadirachta indica*-based biopesticides in replacing synthetic toxic pesticides. Frontiers in Plant Science, **8**:610.

Chowhan, N.; Singh, H.P.; Batish, D.R.; Kaur, S.; Ahuja, N. and Kohli, R.K. (2013). β-Pinene inhibited germination and early growth, involving membrane peroxidation. Protoplasma, **250**(3):691-700.

Cloyd, R.A. and Cycholl, N.L. (2002). Phytotoxicity of selected insecticides on greenhouse-grown herbs. Hort Science, **37**(4):671-672.

Codex Alimentarius Commission (CAC) (1993). Codex Alimentarius: Pesticide Residues in Food and Feed. FAO/WHO, Rome.

Corcos, D.; Mazzon, L.; Cerretti, P.; Mei, M.; Giussani, E.; Drago, A. and Marini, L. (2020). Effects of natural pyrethrum and synthetic pyrethroids on the tiger mosquito, *Aedes albopictus* (Skuse) and non-target flower-visiting insects in urban green areas of Padua, Italy. International Journal of Pest Management, **66**(3):215-221.

Damalas, C.A. and Koutroubas, S.D. (2016). Farmers' exposure to pesticides: Toxicity types and ways of prevention. Toxics, **4**(1):1.

Dayan, F.E.; Cantrell, C.L. and Duke, S.O. (2009). Natural products in crop protection. Bioorganic and Medicinal Chemistry, **17**(12):4022-4034.

El Hadrami, A.; Adam, L.R.; El Hadrami, I. and Daayf, F. (2010). Chitosan in plant protection. Marine Drugs, **8**(4):968-987.

Emwas, A. H., Roy, R., McKay, R. T., Tenori, L., Saccenti, E., Gowda, G. N. and Wishart, D. S. (2019). NMR spectroscopy for metabolomics research. Metabolites, **9**(7):123.

Esparza-Díaz, G.; López-Collado, J.; Villanueva-Jiménez, J.A.; Osorio-Acosta, F.; Otero-Colina, G. and Camacho-Díaz, E. (2010). Azadirachtin concentration, insecticide efficacy and phytotoxicity of four neem *Azadirachta indica* A. Juss. extracts, pp:821-833.

European Commission (2005). Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin. Official Journal of the European Union, **L70**:1-16.

European Commission (2009). Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market. Official Journal of the European Union, **L309**:1-50.

European Medicines Agency (2008). Guideline on specifications: Test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products. EMA Committee on Herbal Medicinal Products (HMPC), London.

European Medicines Agency (EMA) (2008). Guideline on quality of herbal medicinal products/traditional herbal medicinal products. EMA/HMPC.

European Medicines Agency (EMA) (2013). Guideline on Specifications: Test Procedures and Acceptance Criteria for Herbal Substances, Herbal Preparations and Herbal Medicinal Products/Traditional Herbal Medicinal Products (EMA/HMPC/201116/2005 Rev.1). London, UK.

Fathima, S.K.; Bhat, S. and Girish, K. (2009). Efficacy of some essential oils against *Phomopsis azadirachtae*: The incitant of die-back of neem. Journal of Biopesticides, **2**:157-160.

Food Safety and Standards Authority of India (FSSAI) (2011). Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011. Government of India.

Giunti, G.; Benelli, G.; Palmeri, V.; Laudani, F.; Ricupero, M. and Ricciardi, R. (2022). Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates. Biological Control, **176**:105071.

Godard, K.A.; White, R. and Bohlmann, J. (2008). Monoterpene-induced molecular responses in *Arabidopsis thaliana*. Phytochemistry, **69**:1838-1849.

Grana, E.; Díaz-Tielas, C.; López-González, D.; Martínez-Peñalver, A.; Reigosa, M.J. and Sánchez-Moreiras, A.M. (2016). The plant secondary metabolite citral alters water status and prevents seed formation in *Arabidopsis thaliana*. Plant Biology, **18**:423-432.

Grana, E.; Sotelo, T.; Díaz-Tielas, C.; Araniti, F.; Krasuska, U.; Bogatek, R.; Reigosa, M.J. and Sánchez-Moreiras, A.M. (2013). Citral induces auxin and ethylene-mediated malformations and arrests cell division in Arabidopsis thaliana roots. Journal of Chemical Ecology, **39**:271-282.

Grimalt, S.; Thompson, D.G.; Coppens, M.; Chartrand, D.T.; Shorney, T.; Meating, J. and Scarr, T. (2011). Analytical study of azadirachtin and 3-tigloylazadirachtol residues in foliage and phloem of hardwood tree species by liquid chromatography–electrospray mass spectrometry. Journal of Agricultural and Food Chemistry, **59**(15):8070-8077.

Handa, S.K. and Dewan, R.S. (1974). Evaluation of dillapiole and dihydrodillapiole as synergists for pyrethrins in dust formulations. Pyreth Post, **13**:45-46.

Hollingsworth, R.G. (2005). Limonene, a citrus extract, for control of mealybugs and scale insects. Journal of Economic Entomology, **98**(3):772-779.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. Biological research, **52**:314.

Isangadighi, G. E., Obahor, G., Ekanem, M. E., Judith, U. O., Paul, C. J., Adedamola, S. B. and Ozohili, L. I. (2024). Machine learning-based prediction of bioaccumulation and ecotoxicity of emerging contaminants in aquatic ecosystems. Journal of Systematic, Evaluation and Diversity Engineering, **10**:220-233.

Isman, M.B. (2000). Plant essential oils for pest and disease management. Crop Protection, **19**:603-608.

Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, **51**(1):45-66.

Isman, M.B. (2020). Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. Phytochemistry Reviews, **19**:235-241.

Johnson, S. and Dureja, P. (2002). Effect of surfactants on persistence of azadirachtin-A (neem-based pesticide). Journal of Environmental Science and Health Part B, **37**(1):75-80.

Jordan, S.A.; Cunningham, D.G. and Marles, R.J. (2010). Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment. Toxicology and Applied Pharmacology, **243**(2):198-216.

Jouini, A.; Verdeguer, M.; Pinton, S.; Araniti, F.; Palazzolo, E.; Badalucco, L. and Laudicina, V.A. (2020). Potential effects of essential oils extracted from Mediterranean aromatic plants on target weeds and soil microorganisms. Plants, **9**:1289.

Kah, M.; Beulke, S.; Tiede, K. and Hofmann, T. (2013). Nanopesticides: State of knowledge, environmental fate, and exposure modelling. Critical Reviews in Environmental Science and Technology, **43**(16):1823-1867.

Kah, M.; Kookana, R.S.; Gogos, A. and Bucheli, T.D. (2018). A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. Nature Nanotechnology, **13**(8):677-684.

Kavithakumari, N.; Vastrad, A.S. and Basavana Goud, K. (2009). Evaluation of insecticides for the management of eucalyptus gall wasp, *Leptocy beinvasa* Fisher and La Salle. Indian Journal of Entomology, **71**:267-269.

Kordali, S.; Cakir, A.; Ozer, H.; Cakmakci, R.; Kesdek, M. and Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresource Technology, **99**(18):8788-8795.

Kumar, R.; Kaur, R.; Ranout, A.S. and Nadda, G. (2025). A review on novel formulations and delivery systems of botanical insecticides for enhanced efficacy. Neotropical Entomology, **54**(1):81.

Lengai, G.M.W.; Muthomi, J.W. and Mbega, E.R. (2020). Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Scientific African, **7**:e00239.

Li, K.; Xing, R.; Liu, S. and Li, P. (2020). Chitin and chitosan fragments are responsible for plant elicitors and growth stimulators. Journal of Agricultural and Food Chemistry, **68**(44):12203-12211.

Ling, N. (2003). Rotenone: A review of its toxicity and use for fisheries management. Science for Conservation, **211**:1-40.

Liu, S.; Zhao, J.; Hamada, C.; Cai, W.; Khan, M.; Zou, Y. and Hua, H. (2019). Identification of attractants from plant essential oils for *Cyrtorhinus lividipennis*, an important predator of rice planthoppers. Journal of Pest Science, **92**:769-780.

Loke, W.H.; Heng, C.K.; Basirun, N. and Mardi, A.R. (1992). Non-target effects of neem (*Azadirachta indica* A. Juss) on *Apanteles plutellae* Kurdj., Cabbage, Sawi and Padi, pp:108-110.

Lucas, G.C.; Alves, E.; Pereira, R.B.; Zacaroni, A.B.; Perina, F.J. and de Souza, R.M. (2012). Indian clove essential oil in the control of tomato bacterial spot. Journal of Plant Pathology, **94**:45-51.

Marcec, M.J.; Gilroy, S.; Poovaiah, B. and Tanaka, K. (2019). Mutual interplay of Ca^{2+} and ROS signaling in plant immune response. Plant Science, **283**:343-354.

Massimo, E. Maffei (2012). Monoterpenoid plant-plant interactions upon herbivory. Current Bioactive Compounds, **8**:65-70.

Møller, I.M. (2001). Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annual Review of Plant Physiology and Plant Molecular Biology, **52**:561-591.

Mordue (Luntz), A.J. and Nisbet, A.J. (2000). Azadirachtin from the neem tree Azadirachta indica: Its action against insects. Anais da Sociedade Entomológica do Brasil, **29**(4):615-632.

Moreira da Silva, I.; Alvarenga Soares, M.; De Souza Tavares, W.; Dos Santos, A.; Serrão, J.E.; José Vinha Zanuncio, A.; Frederico Wilcken, C.; Cola Zanuncio, J. and Sigueyuki Sediyama, C. (2020). Toxicity of essential oils to *Diaphania hyalinata* (Lepidoptera: Crambidae) and selectivity to its parasitoid *Trichospilus pupivorus* (Hymenoptera: Eulophidae). Journal of Economic Entomology, **113**:2399-2406.

Natal, C.M.; Fernandes, M.J.G.; Pinto, N.F.; Pereira, R.B.; Vieira, T.F.; Rodrigues, A.R.O. and Gonçalves, M.S.T. (2021). New carvacrol and thymol derivatives as potential insecticides: Synthesis, biological activity, computational studies and nanoencapsulation. RSC Advances, **11**(54):34024-34035.

Newman, D.J. and Cragg, G.M. (2020). Natural products as sources of new drugs over nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, **83**(3):770-803.

Pan, Q.; Mustafa, N.R.; Tang, K.; Choi, Y.H. and Verpoorte, R. (2016). Monoterpenoid indole alkaloids biosynthesis and its regulation in Catharanthus roseus: A literature review from genes to metabolites. Phytochemistry Reviews, **15**(2):221-250.

Paspati, A.; Karakosta, E.; Balanza, V.; Rodríguez-Gómez, A.; Grávalos, C.; Cifuentes, D.; Koukaki, A.; Stavrakaki, M.; Roditakis, E. and Bielza, P. (2023). Effects of novel and commercial phytochemicals on beneficial arthropods. Crop Protection, **174**:106381.

Patil, S.J.; Patel, B.R. and Chaudhari, R.K. (2011). Efficacy of synthetic and botanical insecticides against aphid, *Aphis gossypii* Glover, in isabgol crop. Pestology, **35**:32-34.

Paul, A.; Visakh, N.U.; Pathrose, B.; Mori, N.; Baeshen, R.S. and Shawer, R. (2024). Exploring the chemical characterisation and insecticidal activities of *Curcuma angustifolia* Roxb. leaf essential oils against three major stored product insects. Saudi Journal of Biological Sciences, **31**(6):103986.

Pavela, R. (2016). History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects: A review. Plant Protection Science, **52**(4):229-241.

Pavela, R. and Benelli, G. (2016). Essential oils as eco-friendly biopesticides? Challenges and constraints. Trends in Plant Science, **21**(12):1000-1007.

Pereira, V.; Castilho, P.C. and Pereira, J.A. (2025). Analysis of the environmental impact of botanical pesticides in soil. Agriculture, **15**(10):1053.

Perina, F.J.; De Andrade, C.C.L.; Moreira, S.I.; Nery, E.M.; Ogoshi, C. and Alves, E. (2019). *Cinnamomum zeylanicum* oil and trans-cinnamaldehyde against Alternaria brown spot in tangerine: Direct effects and induced resistance. Phytoparasitica, **47**:575-589.

Price, N.R. and Watkins, R.W. (2003). Quantitative structure-activity relationships (QSAR) in predicting the environmental safety of pesticides. Pesticide Outlook, **14**(3):127-129.

Rabeh, K., Hnini, M., and Oubohssaine, M. (2025). A comprehensive review of transcription factor-mediated regulation of secondary metabolites in plants under environmental stress. Stress Biology, **5**(1):15.

Rai, A., Saito, K., and Yamazaki, M. (2017). Integrated omics analysis of specialised metabolism in medicinal plants. The Plant Journal, **90**(4):764-787.

Ramakrishna Mission (2008). Assessment of Bioactivity of Some Locally Available Medicinal and Aromatic Plants in Management of Pests and Diseases of Medicinal Plants in West Bengal. Medicinal Plants Institute, Ramakrishna Mission and Department of Food Processing Industries, Government of West Bengal, Kolkata, West Bengal, India.

Ravikumar, A.; Rajendran, R.; Chinniah, C.; Irulandi, S. and Pandi, R.J. (2008). Evaluation of certain organic nutrient sources against mealy bug, *Coccidohystrix insolitus* (Green) and the spotted leaf beetle, *Epilachnavigintiocto punctata* Fab. on aswagandha, *Withamia somnifera* Duval. Journal of Biopesticides, **1**:28-31.

Regnault-Roger, C. (2012). Botanicals in pest management. In: Integrated pest management: principles and practice (pp:119-132). Wallingford, UK: CABI.

Riedlmeier, M., Ghirardo, A., Wenig, M., Knappe, C., Koch, K., Georgii, E. and Vlot, A. C. (2017). Monoterpenes support systemic acquired resistance within and between plants. The Plant Cell, **29**(6):1440-1459.

Roy, K.; Kar, S. and Das, R.N. (2015). A primer on QSAR/QSPR modelling: Fundamental concepts. Springer.

Sarmah, K.; Anbalagan, T.; Marimuthu, M.; Mariappan, P.; Angappan, S. and Vaithiyanathan, S. (2025). Innovative formulation strategies for botanical and essential oil-based insecticides. Journal of Pest Science, **98**(1):1-30.

Seenivasan, N. (2011). Bioefficacy of anti-nematic plants against root-knot nematode in medicinal coleus. Journal of Ecofriendly Agriculture, **6**:92-96.

Shaki, F., Amirkhanloo, M. and Chahardori, M. (2024). The future and application of artificial intelligence in toxicology. Asia Pacific Journal of Medical Toxicology, **13**(1):21.

Sharma, J.R.; Amrate, P.K. and Gill, B.S. (2010). Leaf soft disease of *Aloe barbadensis* and its management. Journal of Research Punjab Agricultural University, **47**:22-24.

Shreedevasena, S.; Kavya, N.; Reddy, N.K. and Patel, P.S. (2024). Understanding the interplay: Medicinal plants and biotic stress. In: Ethnopharmacology and OMICS Advances in Medicinal Plants Volume 1: Uncovering Diversity and Ethnopharmacological Aspects, Springer Nature Singapore, Singapore, pp. 259-284.

Singh, A.K.; Ganapathysubramanian, B.; Sarkar, S. and Singh, A. (2018). Deep learning for plant stress phenotyping: Trends and future perspectives. Trends in Plant Science, **23**(10):883-898.

Singh, P. and Pandey, A.K. (2018). Perspective of essential oils of the genus Mentha as biopesticides: A review. Frontiers in Plant Science, **9**:1295.

Soares, M.A.; Campos, M.R.; Passos, L.C.; Carvalho, G.A.; Haro, M.M.; Lavoir, A.V.; Biondi, A.; Zappalà, L. and Desneux, N. (2019). Botanical insecticide and natural enemies: A potential combination for pest management against *Tuta absoluta*. Journal of Pest Science, **92**:1433-1443.

Souri, M.K. and Bakhtiarizade, M. (2019). Biostimulation effects of rosemary essential oil on growth and nutrient uptake of tomato seedlings. Scientia Horticulturae, **243**:472-476.

Sridhar, Y. (2011). Field evaluation of different insecticides against aonla aphid, Lengai *Schoutedenia emblica*. Indian Journal of Plant Protection, **39**:35-37.

Sukegawa, S.; Shiojiri, K.; Higami, T.; Suzuki, S. and Arimura, G.I. (2018). Pest management using mint volatiles to elicit resistance in soy: Mechanism and application potential. Plant Journal, **96**:910-920.

Tanner, C. M., Kamel, F., Ross, G. W., Hoppin, J. A., Goldman, S. M., Korell, M. and Langston, J. W. (2011). Rotenone, paraquat, and Parkinson's disease. Environmental Health Perspectives, **119**(6):866.

Tomizawa, M. and Casida, J.E. (2005). Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annual Review of Pharmacology and Toxicology, **45**:247-268.

United States Environmental Protection Agency (USEPA) (2007). Reregistration Eligibility Decision (RED) for Rotenone. Office of Prevention, Pesticides and Toxic Substances, Washington DC.

USEPA (2007). Reregistration Eligibility Decision (RED) for Rotenone. United States Environmental Protection Agency.

Varadharajan, V., Rajendran, R., Muthuramalingam, P., Runthala, A., Madhesh, V., Swaminathan, G. and Ramesh, M. (2025). Multi-omics approaches against abiotic and biotic stress: A review. Plants, **14**(6):865.

Vekemans, M.C. and Marchand, P.A. (2020). The fate of biocontrol agents under the European phytopharmaceutical regulation: How this regulation hinders the approval of botanicals as new active substances. Environmental Science and Pollution Research, **27**(32):39879-39887.

Werrie, P.Y.; Durenne, B.; Delaplace, P. and Fauconnier, M.L. (2020). Phytotoxicity of essential oils: Opportunities and constraints for the development of biopesticides. A review. Foods, **9**(9):1291.

Weston, D.P.; Holmes, R.W.; You, J. and Lydy, M.J. (2005). Aquatic toxicity due to residential use of pyrethroid insecticides. Environmental Science and Technology, **39**(24):9778-9784.

Wolfender, J.L.; Marti, G.; Thomas, A. and Bertrand, S. (2015). Current approaches and challenges for the metabolite profiling of complex natural extracts. Journal of Chromatography A, **1382**:136-164.

World Health Organisation. (2009). The use of herbal medicines in primary health care (No. SEA-HSD-322). WHO Regional Office for South-East Asia.

World Health Organisation. (2007). WHO guidelines for assessing the quality of herbal medicines with reference to contaminants and residues. World Health Organisation.

Yan, S., Bhawal, R., Yin, Z., Thannhauser, T. W., and Zhang, S. (2022). Recent advances in proteomics and metabolomics in plants. Molecular Horticulture, **2**(1):17.

Yi, C. G., Hieu, T. T., Lee, S. H., Choi, B. R., Kwon, M. and Ahn, Y. J. (2016). Toxicity of Lavandula angustifolia oil constituents and spray formulations to insecticide susceptible and pyrethroid resistant *Plutellaxy lostella* and its endoparasitoid *Cotesia glomerata*. Pest Management Science, **72**(6):1202-1210.

Citation **Chakati Santhosh, Ettiappan Sumathi, Sampathrajan Vellaikumar, Kannan Senguttuvan and Alagarswamy Senthil (2026). Phytotoxicology of botanical pesticides in medicinal crops: Mechanisms, multitrophic effects, and residue implications for high value phytomedicines. Ann. Phytomed., 15(1):157-173, http://dx.doi.org/10.54085/ap.2026.15.1.14.**